THE BASAL GANGLIA:

A MAMMALIAN AND AVIAN PERSPECTIVE

Thesis submitted for the degree of

DOCTOR OF PHILOSOPHY

by

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in

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April 1981

Declaration

I declare that this thesis contains no material which has been submitted or accepted for the award of any other degree or diploma in any university. Most of the research presented was performed in collaboration with other investigators. Only papers in which I am first author are in the text, those of which I am second author are included in the appendices. All investigators involved with each study are fully acknowledged within the thesis.

Paul R. Sanberg

Acknowledgements

My sincerest appreciation is extended to my supervisors, Richard F. Mark and William P. Bellingham, and my mentors, Hans C. Fibiger, Edith G. McGeer and Michele Pisa, without whose unending cooperation the research presented here would not have been possible. A special thank you to Ian G. Morgan for his more than generous help as an advisor, teacher, editor and friend. Thanks also to my many coauthors, collaborators and collegues for the many hours of science that we enjoyed together. I thank my Australian friends for making a stranger in a strange land not feel so strange. Finally to Karen for putting up with my long hours and caring enough to see us through the hard times.

DEDICATED TO KAREN, MOLLY, BERNIE

AND THE REST OF MY FAMILY

ABSTRACT

The roles which the largest part of the basal ganglia, the striatum, plays in motor, psychological and ingestive behaviours were studied in rats. Animals with selective lesions in the striatum by kainic acid and with control operations were compared in various behavioural experiments. Alterations in striatal lesioned rats were found in 1) spontaneous locomotion as well as dopaminergic and cholinergic influenced locomotor activity and catalepsy, 2) exploration, spatial alternation and serial memory performance, and 3) feeding and drinking behaviours, under various types of regulatory challenges, and body weight regulation. These alterations appeared similar in many aspects to symptoms of Huntington's disease in humans, and supported the suggestion from biochemical and anatomical studies that rats with kainic acid lesions of the striatum may provide a useful animal model for the disease. Body weight and dietary factors were also studied in Huntington's disease patients, since the rats with kainic acid-induced striatal degeneration suggested that impairments in these behaviours may be found. Huntington's disease patients showed consistent deficits in body weight and ingestive behaviour.

The avian 'corpus striatum' is usually referred to as the whole forebrain of the bird. However, embryological, anatomical and histochemical studies reviewed here suggest that only the basal part (paleostriatum) of the telencephalon can be considered to be homologous to the mammalian basal ganglia. In a series of behavioural experiments, the effect of dopaminergic and cholinergic drugs on chicken motor activity and tonic immobility were studied. As with mammals, pharmacological manipulation of these transmitter systems influenced motor behaviour in similar ways. Telencephalic injections of

cycloheximide or glutamate, or small stereotaxic injections of kainic acid into the paleostriatum, were also found to alter various types of drug-induced motor behaviour and body weight regulation. Furthermore, the previously reported permanent retarded learning in chickens after telencephalic injections of cycloheximide or glutamate were found to be due to enhanced emotional reactions and convulsive behaviour in these birds, at least in part. The behavioural and pharmacological findings with chickens support a parallel between the paleostriatal complex of the forebrain in birds and the basal ganglia in mammals.

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CHAPTER ONE

OVERVIEW OF THESIS

1.1 THE MAMMALIAN BASAL GANGLIA

1.1.1 *Gross neuroanatomy*

Over the years, the term "basal ganglia" has included many different subcortical nuclei in mammalian cerebral hemispheres and even the thalamus. It seems that the term is now reserved for several gray masses embedded in the white matter of each cerebral hemisphere and in the upper brainstem. The basal ganglia include the caudate nucleus, putamen, globus pallidus, claustrum, subthalamic nucleus and substantia nigra (Carpenter, 1976). Morphologically, the amygdaloid nucleus is part of the telencephalic basal ganglia, however it is a functional component of the olfactory and limbic systems (Isaacson, 1974). At present, basal ganglia refer to motor nuclei that are closely related functionally and in clinical and pathological contexts. The basal ganglia constitutes the major part of the complex extrapyramidal motor system, which works in combination with the corticobulbar and corticospinal tracts composing the pyramidal motor system (Barr, 1974). The caudate, putamen, globus pallidus and claustrum have a streaked appearance in myelin-stained sections and are therefore called, collectively, the corpus striatum. Because of their lens-like shape, the putamen and globus pallidus together are often referred to as the lenticular nucleus. More meaningful relationships however, can be derived from the ontogenetic and phylogenetic development of the basal ganglia.

The mammalian caudate nuclei and putamen have identical gross appearances and histological structures. The cells of these nuclei are densely packed, are not organised

in laminar arrays or other special arrangements, and are of two types. The small achromatic neurons probably outnumber large multipolar neurons by at least 60:1 (Carpenter, 1976). The small neurons usually have spiny dendrites that radiate into a spherical space and short axons (Golgi type II neurons). The large neurons are aspinous and usually have bulbous cell bodies with well-formed Nissl granules, myelinated axons which emit collaterals, and smooth dendrites that extend into an elongated field. These neurons probably give rise to striatal efferents (Fox, 1976). Both the caudate and putamen have appeared relatively recently in terms of the phylogenetic development of the brain. They are both derived from a thickening of the basal region of the lateral telencephalic vesicle, known as the striatal ridge. In humans, the medial portions of this ridge are carried dorsally and caudally over the diencephalon in a curvature parallel to the lateral ventricle, forming the caudate nucleus. The putamen is formed from the larger lateral portion of the striatal premordium (Hamilton and Mossman, 1972). Together these two nuclei are called the neostriatum or simply the striatum. The phylogenetically older globus pallidus develops from the basal plate of the neural tube and is referred to as the paleostriatum or pallidum.

The globus pallidus is medial to the putamen, and is divided by a medial medullary lamina into the medial and lateral globus pallidus. The medial globus pallidus is smaller than the lateral globus pallidus, and is further divided by an accessory medullary lamina into inner and outer portions (Carpenter, 1976).

The neurons of the globus pallidus are ovoid or polygonal in shape and have long, thick relatively smooth dendrites. There appear to be no significant differences in the large cells of the lateral and medial globus pallidus (Fox and Rafols, 1976). The globus pallidus contains a rich afferent plexus of fine fibers that invest the perikarya and their long dendrites.

In humans the internal capsule separates the medially placed caudate nucleus and the laterally placed putamen, except at their anterior ventral extremes. In rodents, the neostriatal nuclei are not separated, and the internal capsule traverses them. The claustrum is just medial to the insular cortex and is separated from the putamen by the external capsule. The subthalamic nucleus is medial to the internal capsule and ventral to the thalamus. Embryological studies suggest that this nucleus may be derived from the dorsocaudal part of the lateral hypothalamic cell columns (Carpenter, 1976). Golgi studies have revealed two principal cell types of neurons within the subthalamic nucleus, those with radiating dendrites and occasional delicate spines, and elongated neurons with polar dendrites (Rafols, 1975).

The substantia nigra, so called because it is darkly pigmented in freshly cut brain due to the melanin in the large neurons, is located dorsomedial to the cerebral peduncle. The substantia nigra is commonly divided into two parts, the pars compacta and the pars reticulata. The pars compacta is a cell-rich area composed of large, pigmented cells, whereas the pars reticulata is a cell-poor area. Golgi studies have revealed that dendrites of nigral neurons vary in size,

branch once or twice, and appear to have specific orientations (Carpenter, 1976). Considerable overlap of the dendritic fields of most nigral neurons in the pars reticulata is apparent. This area appears to represent the principal site of afferent synaptic articulations (Grofová and Rinvik, 1970). Within the substantia nigra there are small interneurons with short axons (Fox and Rafols, 1976). In the rodent substantia nigra, three types of neurons have been described (Gulley and Wood, 1971). There are large neurons distributed exclusively in the pars reticulata, medium-sized neurons in the pars compacta, and small cells with short axons found in both the pars compacta and pars reticulata. Many investigators have recognised similarities between the pars reticulata and the globus pallidus; for both these areas receive the output from the striatum (Fox and Rafols, 1976).

1.1.2 *Biochemical neuroanatomy*

Our knowledge of the connections between different areas of the basal ganglia is in many ways still incomplete. Yet, over the last few years a great deal of information has been obtained, not only about the anatomical details of pathways, but also about the neurotransmitters used in basal ganglia pathways. Extensive reviews of these data have recently been published (Carpenter, 1976; Dray, 1979; McGeer *et al.*, 1979) and the details will not be reviewed here, but there is a general pattern of connections that is important.

Essentially, the major connections through the basal

ganglia are from the cerebral cortex, to striatum, to globus pallidus to thalamus, and back to motor and premotor cerebral cortex. In addition, the striatum is influenced by various thalamic and brain stem nuclei. These include afferent tracts from the substantia nigra, thalamus, raphe nucleus and locus coeruleus, and efferent tracts back to the substantia nigra. The following are brief descriptions of basal ganglia pathways in which at least one possible transmitter has been suggested.

Corticostriate Tract (Glutamate)

Virtually all parts of the cerebral cortex project to the striatum in a topographic manner. The frontal cortex sends efferent fibers to the head of the caudate and anterior putamen. The posterior portions of the cortex project to the caudal portion of the striatum. No part of the striatum, however, is under the sole influence of one functional area of the neocortex, but the projection from the sensorimotor cortex is much more substantial than that from the occipital cortex (Kemp and Powell, 1970). Corticostriate fibers end predominantly on the dendritic spines of spiny striatal neurons (Kemp and Powell, 1970; Adinolfi and Pappas, 1968). Both McGeer *et al.* (1977) and Divac *et al.* (1977) have found that lesions of this pathway resulted in a 40 - 50% drop in high affinity glutamate uptake in the synaptosomal fraction of the ipsilateral striatum. Since in the rat, corticostriate projections from the sensorimotor cortex are bilateral (Carman *et al.*, 1965), it is likely that a larger drop in uptake would

have been achieved with bilateral lesions. Also Kim *et al.* (1977) demonstrated a drop in glutamate levels in the striatum but no changes in the levels of other amino acids, including aspartate, following corticostriate lesions were observed. It is controversial whether there is a striocortical pathway, however it is noteworthy that Jayaraman (1980) has recently presented convincing evidence for a striocortical tract in cats using horseradish peroxidase tracing techniques.

Striopallidal tract (GABA and Enkephalin)

A massive pallidal input comes from the striatum which is organised in a rather simple topographical manner. Basically, the head of the caudate projects to dorsal and rostral parts of the pallidum, whereas the putamen projects to ventral and caudal parts (Szabo, 1962, 1967, 1970). GABA and enkephalin have been identified as transmitters in this projection (McGeer *et al.*, 1979).

Strionigral tract (GABA and Substance P)

Fibers from the head of the caudate nucleus project to the rostral third of the substantia nigra and have a mediolateral correspondence. Putamen efferents project to the caudal two-thirds of substantia nigra, with dorsal regions of the putamen related to lateral parts of the substantia nigra, and ventral regions related to the medial parts (Szabo, 1962, 1967, 1970). This projection has been suggested to use GABA as a transmitter. The GABAergic striopallidal and strionigral pathways may be axonal branches on the same descending system (Kim *et al.*, 1977 ; Fonnum *et al.*, 1978). Evidence for substance P as transmitter

in this pathway has been obtained by Kanazawa *et al.* (1977) and Mroz *et al.* (1977) using lesioning techniques. In addition, there appears to be a separate pallidol-nigral substance P containing pathway (Jessell *et al.*, 1978).

Nigrostriate tract (Dopamine)

Large cells of the substantia nigra, pars compacta give rise to diffuse nigrostriatal projections that primarily use dopamine as the transmitter for the striatum (Ungerstedt, 1971). This is supported with anatomical findings that striatal lesions produce retrograde cell changes and cell loss in the substantia nigra, pars compacta (Bedard *et al.*, 1969).

Dorsal raphe-striate tract (Serotonin)

The B7, B8 and mainly the B9 cell groups in the dorsal raphe give rise to a serotoninergic pathway to the striatum (Fuxe and Johnsson, 1974).

Locus coeruleus-striate tract (noradrenaline)

The locus coeruleus sends a minor noradrenergic input to the striatum (Lindvall and Bjorkland, 1978).

Other tracts

Three other pathways which appear to use GABA as a transmitter are the projections from a) the intrapeduncular nucleus (primate medial globus pallidus) to the lateral habenula (Nagy *et al.*, 1978), b) the pars compacta of the substantia nigra to the superior colliculus (Vincent *et al.*, 1978) and c) the

globus pallidus to the subthalamic nucleus (Fonnum *et al.*, 1978).

Striatal interneurons

The striatum contains large numbers of cholinergic interneurons (Butcher, 1977). This has been shown by the high levels of acetylcholine and choline acetyltransferase, and by the lack of effect of lesions of the afferent and efferent pathways on cholinergic indices (McGeer *et al.*, 1971). In addition, positive identification of choline acetyltransferase in interneurons has been obtained by immunohistochemistry and electron microscopic techniques (Hattori *et al.*, 1976; McGeer *et al.*, 1979).

There is evidence for GABAergic interneurons within the striatum (McGeer and McGeer, 1975), in addition to the GABAergic efferent described above. Lesion studies have also suggested that angiotensin II may be a transmitter in striatal interneurons (Singh and McGeer, 1978; McGeer *et al.*, 1979).

For many years, it was thought that the basal ganglia was a major relay in the multisynaptic descending pyramidal pathways leading from cortex to spinal cord and brainstem segments (see Patton *et al.*, 1976). From the pattern of the complex connections which we are finally beginning to understand, it is clear that the basal ganglia do not affect motorneuron pools, but primarily influence the motor cortex via thalamic relays. It is likely that the integration of basal ganglia and cerebellum occurs at thalamic level, since the ventral lateral nucleus of the thalamus is a major relay for cerebellar and substantia nigra output (Barr, 1974; Clark, 1975).

1.2 THE AVIAN BASAL GANGLIA

In contrast to the laminar organization of the mammalian cortical mantle, the avian telencephalon has only a very small region of clearly laminated pallium (the corticoid, parahippocampal and Wulst regions) (Benowitz, 1980). Because the remaining bulk of the hemisphere has a striated appearance, Ariëns Kappers *et al.* (1936) regarded the nonlaminar regions of the avian telencephalon as elaborations of the mammalian basal ganglia. The suffix "-striatum" was thus ascribed to each region. These include the hyperstriatum, neostriatum, ectostriatum, archistriatum and paleostriatum. The region thought to be directly comparable to the caudate nucleus and putamen (neostriatum) in mammals was also named neostriatum (Karten, 1969). Although the nomenclature of Ariëns Kappers *et al.* (1936) is still in use, over the past few years embryological, anatomical and neurochemical evidence has suggested that only the paleostriatum is truly homologous to the basal ganglia (Benowitz, 1980; Brauth *et al.*, 1974; Goodman and Azzaro, 1978; Karten, 1969; Karten and Dubbeldam, 1973; Kitt and Brauth, 1979; Nistico' and Stephenson, 1979). Figure 1 shows the location of the paleostriatum from a saggital view. The embryological origin of the hyperstriatum ventrale, neostriatum, ectostriatum and portions of the archistriatum suggest that they are homologous with portions of the mammalian neocortex (Table 1 and Figure 2). This is also supported by anatomical and histochemical findings (Benowitz, 1980; Cohen and Karten, 1974; Karten, 1969; Karten and Dubbeldam, 1973).

The paleostriatum is positioned in the basolateral wall of the telencephalon and consists of three major subdivisions.

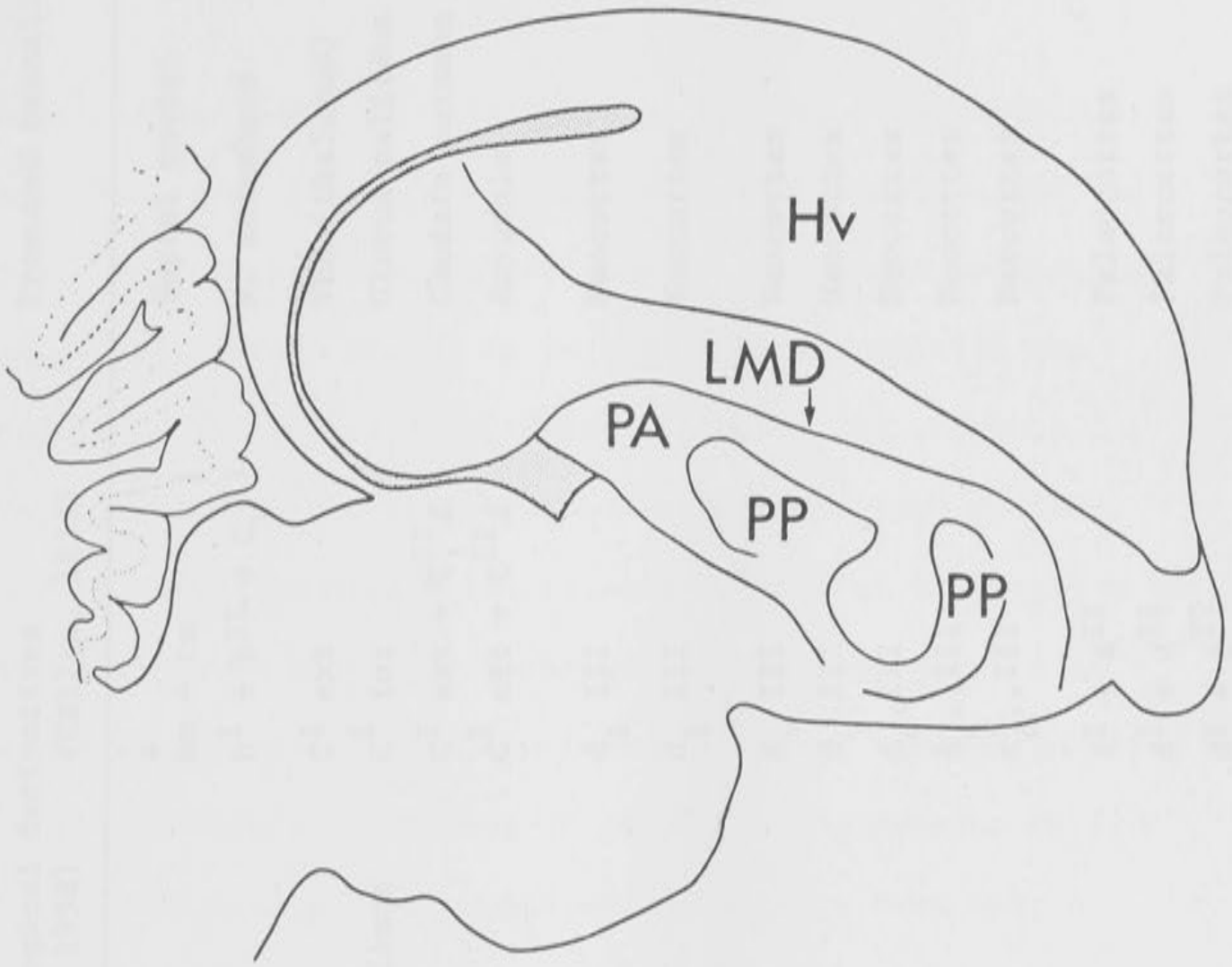


Figure 1. Schematic representation of saggital section of chicken brain to illustrate position of paleostriatal complex. Hv, hyperstriatum ventrale; LMD, lamina medullaris dorsalis; PA, paleostriatum augmentatum; PP, paleostriatum primitivum.

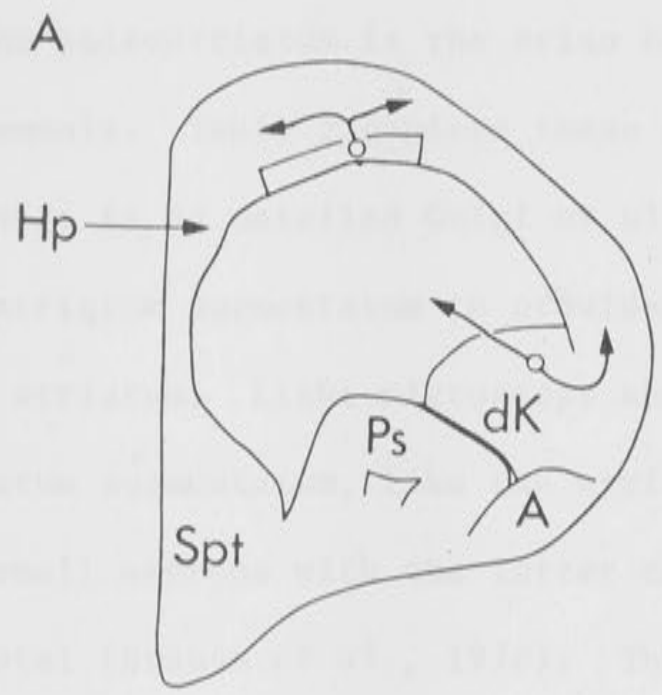
Embryological Origins and Proposed Mammalian Homologies of some Avian Telencephalic Nuclei*

Structure (Ariëns Kappers *et al.*, 1936)	Embryological derivatives (Kuhlenbeck, 1938)	(Källen, 1951)	Presumed mammalian homology
N. lateralis septi	B3 + B4	a)	Septal nuclei
N. medialis septi	B3 + B4	bm + cm)	
N. accumbens	B2	$b^{I} + b^{II} + C_{V}{}^{II}$	N. accumbens
N. basalis	D1	C^{I} ext	VPM (thalamus)
Paleostriatum primitivum	B2 + hypothalamus	C^{I} int	Globus pallidus
Paleostriatum augmentatum	B1 + B2	C^{I} ext + $C^{II}d$	Caudate putamen
Archistriatum mediale, posterior + n.taeniae		C^{I} ext + $C^{II}d$	Amygdala
Ectostriatum	D1	d_{1} III	Neocortex
Archistriatum anterior intermediate, dorsale	D1	d_{1} III	Neocortex
Neostriatum	D1	d_{v} III	Neocortex
Field L	D1	d_{v} III	Neocortex
Hyperstriatum ventrale	D1	$d_{d'}$,III	Neocortex
Hyperstriatum dorsale	D1	$d_{d''}$,III	Neocortex
Hyperstriatum intercalatus superior	D2	$d_{d''}$,III	Neocortex
Hyperstriatum accessorium	D2	$d^{I} + d^{II}$	Paleocortex
Area parahippocampalis	D3	$d^{I} + d^{II}$	Paleocortex
Hippocampal region	D2	$d^{I} + d^{II}$	Paleocortex

*modified from Benowitz (1980)

Figure 2

Hypothetical model of relationships between telencephala of amniotes, and alternate possible routes of developement and migration of telencephalic neurons of the dorsal ventricular ridge (dK). This figure is primarily concerned with the nature of the dorsal ventricular ridge and its possible contribution to the formation of neocortical type systems. Karten (1969) considers the paleostriatum (Ps; neostriatum in mammals) to be basically unchanged in all amniotes. A is a generalized model of amniote telencephalon. Arrows indicate the various possible migrations of neurons. The neurons derived from the dorsal pallial ependyma may migrate over only a restricted field in all amniotes, whereas the neurons arising from the dK may migrate either medially, forming and expanding intraventricular mass, or laterally into the pallium. B is a hypothetical model of the avian brain indicating a major intraventricular development from dK, with only a minimal contribution to the lateral pallium. C is a hypothetical model of the mammalian brain indicating that the majority of the dK derivatives enter the pallium, perhaps mingling with cells of local pallial ependymal origins to form the cortex. Redrawn from Karten (1969). Figure is on next page.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dK
Ps
A
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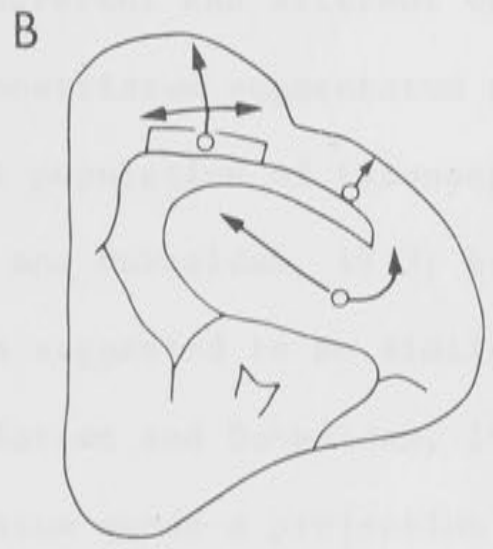
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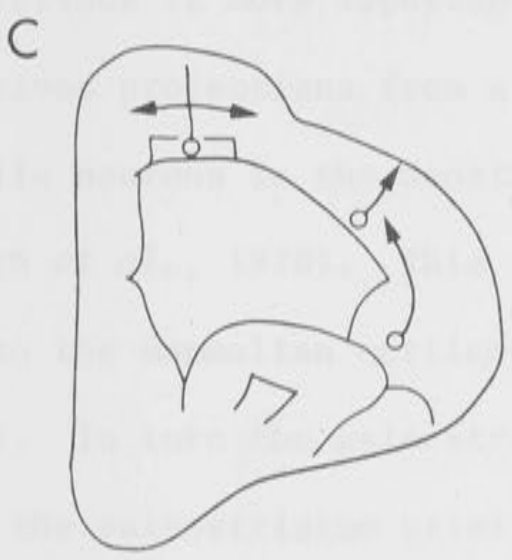
C

These are the paleostriatum augmentatum, paleostriatum primitivum and the nucleus intrapeduncularis. The lobus parolfactorius, positioned on the medial part of the paleostriatum augmentatum is often considered part of the paleostriatum augmentatum. Recent anatomical and histochemical findings support the previous suggestions that the paleostriatum is the avian homologue of the basal ganglia in mammals. Table 2 depicts these comparisons.

As yet there is no detailed Golgi or ultrastructural study of the paleostriatum augmentatum to provide comparison with the mammalian striatum. Light microscopy shows, however, that the paleostriatum augmentatum, like the striatum, is composed of both large and small neurons with the latter constituting about 90% of the total (Brauth *et al.*, 1978). The comparison of the efferent and afferent connections is more important. The paleostriatum augmentatum receives projections from a distinct population of telencephalic neurons in the neostriatum (Karten and Dubbeldam, 1973; Brauth *et al.*, 1978). This tract has been suggested to be similar to the mammalian corticostriate tract (Karten and Dubbeldam, 1973). In turn the paleostriatum augmentatum sends a projection to the paleostriatum primitivum and the nucleus intrapeduncularis (Karten and Dubbeldam, 1973; Brauth *et al.*, 1978). This tract is considered homologous to the striopallidal tract in mammals. Figure 3 comparatively depicts these two connections. In addition, the paleostriatum augmentatum receives extensive projections from the nucleus tegmenti pedunculopontinus of the midbrain, a dopamine-containing cell group (Brauth *et al.*, 1978). The tegmentopaleostriatal

Table 2

Terminology of Corpus Striatum in Mammalian and Avian Brain

MAMMALIAN.

Corpus Striatum = Neostriatum + Paleostriatum

Neostriatum = Striatum = Caudate Nucleus + Putamen

Paleostriatum = Pallidum = Globus Pallidus (medial + lateral)

Lenticular Nucleus = Pallidum + Putamen

AVIAN.

Corpus Striatum = Hyperstriatum + Neostriatum + Ectostriatum
+ Archistriatum + Paleostriatum

Paleostriatum = Paleostriatum Augmentatum + Paleostriatum
Primitivum + Nucleus Intrapeduncularis

MAMMALIAN		AVIAN
Corpus Striatum	=	Paleostriatum
Neostriatum	=	Paleostriatum Primitivum + Nucleus Intrapeduncularis
Lateral Globus Pallidus	=	Paleostriatum Primitivum
Medial Globus Pallidus	=	Nucleus Intrapeduncularis

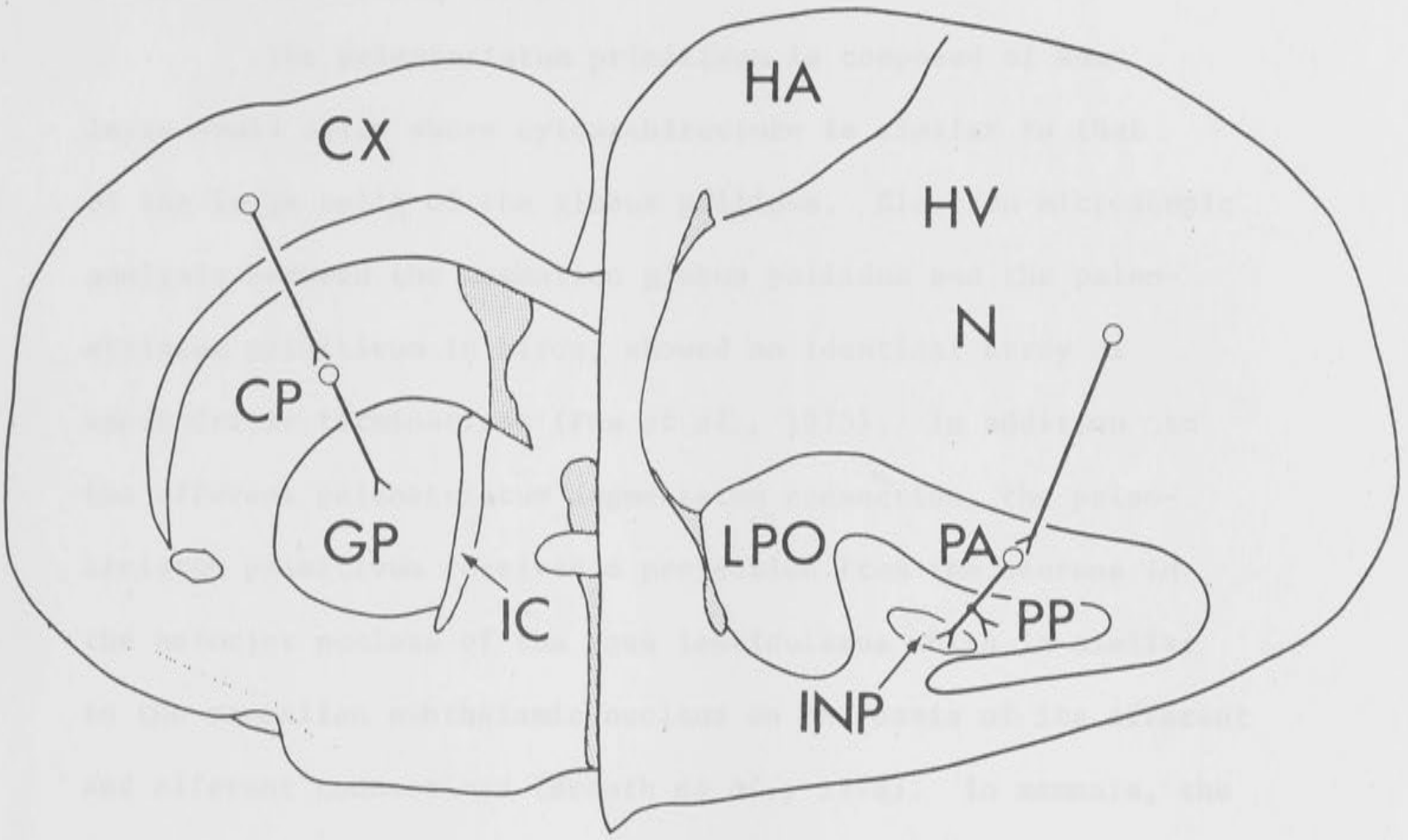

Figure 3. Schematic representation of coronal sections of rat and chicken brains showing avian homologues of corticostriate and striopallidal pathways of mammals. CX, cortex; CP, caudate-putamen (striatum); GP, globus pallidus; IC, internal capsule; HA, hyperstriatum accessorium; HV, hyperstriatum ventrale; N, neostriatum; PA, paleostriatum augmentatum; PP, paleostriatum primitivum; LPO, lobus parolfactorius; INP, nucleus intrapeduncularis.

tract is thought to be homologous to the nigrostriatal tract (Goodman and Azzaro, 1978).

The paleostriatum primitivum is composed of many large ovoid cells whose cytoarchitecture is similar to that of the large cells of the globus pallidus. Electron microscopic analysis between the mammalian globus pallidus and the paleostriatum primitivum in birds, showed an identical array of axodendritic terminations (Fox *et al.*, 1975). In addition to the afferent paleostriatum augmentatum connection, the paleostriatum primitivum receives a projection from the neurons in the anterior nucleus of the ansa lenticularus which is similar to the mammalian subthalamic nucleus on the basis of its afferent and efferent connections (Brauth *et al.*, 1978). In mammals, the globus pallidus receives projections from the subthalamic nucleus (Carpenter, 1976). The avian homologue of the pallido-thalamic tract appears to be the projection from the paleostriatum primitivum to the nucleus dorso-intermedius posterior thalamus (Brauth *et al.*, 1978).

At present, only one transmitter has been assigned to an avian basal ganglia pathway. The paleostriatum augmentatum contains the highest concentration of dopamine in the brain which seems to be released from fibers arising from the nucleus tegmenti pedunculopontinus (Nistico' and Stephenson, 1979). Also, Karten and Dubbeldam (1973) have shown a lot of acetylcholinesterase in the paleostriatum augmentatum. These two biochemical indices are key histochemical features of mammalian striatum (McGeer *et al.*, 1979).

Table 3 summarises the non-developmental evidence supporting the view that the paleostriatal complex is the avian

Table 3

Interconnection Evidence for Avian Basal Ganglia Homologies

Structure in Avian Brain	Probable Homologous Structure in Mammalian Brain	Evidence in Support of Comparability between Avian and Mammalian Neural Structures	Sources
Paleostriatum augmentatum (PA)	Caudate nucleus and putamen	High concentrations of dopamine and acetylcholinesterase; PA cells project to PP.	Karten and Dubbeldam, 1973 Brauth *et al.*, 197
Paleostriatum privitivum (PP)	Lateral globus pallidus	Cytoarchitecture; PP receives projections from PA and generates the ansa lenticularis.	Karten and Dubbeldam, 1973 Brauth *et al.*, 197
Nucleus Intrapeduncularis	Medial globus pallidus Possible extension of rostral thalamic cell groups	Apparent PA afferents Reciprocal connections with TPO	Karten and Dubbeldam, 1973 Brauth *et al.*, 197
Area tempero-parieto-occipitalis of neostriatum (TPO)	Neocortical neurons projecting upon striatum	Efferent projections to PA	Brauth *et al.*, 197
Nucleus tegmenti pedunculo-pontinus	Substantia nigra pars compacta	Position in midbrain; contains dopamine; projects upon PA	Brauth *et al.*, 197
Nucleus spiriformis lateralis thalami	Unknown, however, may be comparable to pars reticulata of substantia nigra	Receives PP efferents; projects upon the optic tectum	Brecha *et al.*, 197 Graybiel and Scal 1975
Ansa lenticularis	Subthalamic nucleus	Reciprocal connections with PP.	Brauth *et al.*, 197
Nucleus dorsointermedius posterior thalami	Ventral tier group of dorsal thalamus	Receives PP projection as well as projection from cerebellum; projects upon the rostral telencephalon	Karten and Dubbeldam, 1973 Brauth *et al.*, 197

Modified from Brauth *et al.* (1978)

homologue of the basal ganglia. Continued evidence that the analogous avian interconnections use the same transmitters as the mammalian basal ganglia connections would be important for a truly homologous comparison. The fact that great strides in our understanding of the biochemical neuroanatomy of the mammalian basal ganglia are being made, should facilitate such analysis in the avian brain. In the final analysis, though, a functional homology should be shown. At present such studies in the avian 'basal ganglia' are extremely rare, in comparison to the extensive work done on the role of the mammalian basal ganglia in behaviour (see overviews of following chapters)

1.3 THE THESIS

The work reported in this Thesis was concerned primarily with elucidating the functions of the largest part of the basal ganglia, the striatum. Data of humans, rats and chickens are presented in order to define striatal function in normal and pathological states, and provide further evidence on the homology between the avian paleostriatum and the mammalian basal ganglia using behavioural studies. The classical role of the striatum is in the regulation of motor behaviour (Martin, 1967) and diseases of this area usually result in motor disturbances (chorea and dyskinesia), characterized by uncontrollable, purposeless movements and other signs. However, more recent findings have demonstrated that the striatum may be involved in psychological behaviour and body weight regulation. Each of the subsequent

four chapters is concerned with studies in one of these aspects. Chapter Two explores the role of the striatum in motor behaviour. Chapter Three examines if the striatum is involved in psychological processes. Chapter Four demonstrates a role for the striatum in feeding, drinking and body weight regulation. Finally, Chapter Five is concerned with human diseases that involve striatal dysfunction. Overviews on each of these aspects are presented at the beginning of each chapter, therefore the details need not plague us here. Nevertheless, there are two techniques used throughout this Thesis which need clarification. They are the kainic acid lesioning technique and the 'retarded' learning syndrome.

1.3.1 *Kainic acid as a tool for lesioning the striatum*

Glutamic acid is a putative neurotransmitter which, when applied iontophoretically, causes neuronal excitation (Johnston *et al.*, 1974). Kainic acid is a conformationally restricted analogue of glutamic acid which is more potent in exciting neurons than glutamic acid (Johnston *et al.*, 1974). For years it has been known that systemic injection of glutamic acid causes neuronal death (Johnston, 1979; Olney, 1971). Similarly, such injections of kainic acid were also shown to be extremely potent in destroying neurons (Olney *et al.*, 1974). Olney *et al.* (1974) originally suggested that kainic acid was toxic because it activated glutamate receptors on neuronal surfaces resulting in prolonged depolarization and death. Thus, the term "excitotoxic" was attributed to this effect (Olney *et al.*, 1974). It was later shown by Olney and his colleagues (1975) that axons of passage were spared after injection of

excitotoxic amino acids. Based on the excitotoxic hypothesis, this was explained in terms of the largely dendrosomal distribution of glutamate receptors (Olney, 1975; Johnston, 1979).

In 1976, Coyle and Schwarcz (1976) and McGeer and McGeer (1976) reported that stereotaxic injections of kainic acid into the rat striatum destroyed neurons located within the striatum, while sparing axons of passage and those projecting into the striatum. Furthermore, glial and vascular systems were not destroyed (Coyle *et al.*, 1978).

The ability of kainic acid to lesion striatal neurons is dependent on a number of factors. McGeer and McGeer (1978) demonstrated that the rate, dose and volume of the kainic acid injection are important parameters. In addition, the strain of the animal (Sanberg *et al.*, 1979) and age (Gaddy *et al.*, 1979) can affect the resulting striatal lesions. But, perhaps the most interesting finding is that the ability of kainic acid to lesion striatal neurons is dependent on the presence of glutamatergic nerve terminals from the corticostriatal tract (Biziere and Coyle, 1978; McGeer *et al.*, 1978). McGeer *et al.* (1978b) postulated two other mechanisms besides Olney's excitotoxic hypothesis to account for the apparent dependency of kainic acid neurotoxicity on the integrity of the cortico-striate system.

The first hypothesis suggests that kainic acid may not act directly on the postsynaptic glutamic acid receptors, but may cause release of glutamatic acid and/or inhibit its reuptake into

nerve endings or glia. The neurotoxicity of kainic acid would be an indirect effect due to abnormally high levels of glutamic acid remaining in the synaptic cleft. In support of this Johnston *et al.* (1979) demonstrated that kainic acid inhibited high affinity glutamic acid uptake. However, recent experiments have suggested that the interaction of kainic acid with presumed glutamatergic pathways is independent of the ongoing electrical activity and neurotransmission within the pathway (Nadler *et al.*, 1981, Sanberg and Creese, 1981), and in addition Nadler *et al.* (1981) have suggested that kainic acid can interact with pathways which are not glutamatergic. Another mechanism of action suggested by McGeer *et al.* (1979b) is that a toxic metabolite of kainic acid is formed in the glutamatergic terminals which then destroys the postsynaptic neurons. As yet, however, there is no evidence of such a metabolite.

It is apparent that kainic acid injections into the striatum of rats or paleostriatum of birds (Rieke, 1980) produce much more selective lesions than electrolytic or coagulative techniques. This is important since it allows, for the first time, behavioural deficits to be attributed to the striatum *per se*, and not to terminating or traversing fiber bundles. Unfortunately, during the course of this Thesis it was noticed by several research groups (Ben-Ari *et al.*, 1978, 1980; Nadler *et al.*, 1981; Schwob *et al.*, 1980, Zaczek and Coyle, 1978) that under certain circumstances localised injections of kainic acid can produce widespread damage, particularly within limbic structures. Schwob *et al.* (1980)

have suggested that this extrastriatal damage might be a result of the toxic influence being "transmitted or at least enhanced by intense electrical activity in axons emanating from the injection site". This was based on their findings that the distant lesions were anatomically related to the local structures. This "intense" electrical activity is most likely epileptiform in nature, since Ben-Ari *et al.* (1979, 1980) and Zaczek and Coyle (1978) have shown that anticonvulsants and anaesthetics can diminish the epileptogenic neurotoxic actions of kainic acid (Nadler *et al.*, 1981) and prevent the distant, but not local neuronal degeneration (Ben-Ari *et al.*, 1979, 1980). In later experiments reported in this Thesis we also found that pretreatment with diazepam protected against the extrastriatal damage (Sanberg, 1980). It is strongly urged that in future studies in which kainic acid is used to elucidate the function of striatal neurons, precautions be taken to avoid extrastriatal pathology.

1.3.2 *Cycloheximide, glutamic acid and retarded learning*

Cycloheximide is an antibiotic which inhibits cytoplasmic ribosomal protein synthesis in the brain. It has been used extensively to give evidence that memory formation is associated with brain protein synthesis (Gibbs and Mark, 1973). However, in 1972, Rogers and Mark reported that injections of cycloheximide into the telencephalon of young chickens produced permanent impairments of learning. The susceptible time period in which cycloheximide had this effect seemed to

coincide with the imprinting period. Rogers and her colleagues have since analysed this phenomenon further, and have obtained some interesting behavioural and biochemical results.

The retarded learning found in these birds was demonstrated by the fact that they failed to reach the control levels of performance on a pebbled floor task; that is, they failed to choose predominantly grain, in preference to pebbles, in the latter half of the 60 pecks of the test (Rogers *et al.*, 1974). Furthermore, sensory input (visual and auditory) within 3h following the injections was required to slow subsequent acquisitions of these behavioural tasks (Rogers and Drennen, 1978), and sensory deprivation or anaesthetics 'protected' against these effects (Drennen, 1977). Other behavioural deficits shown in these birds included a slower habituation rate to visual and auditory stimuli (Rogers *et al.*, 1974), an increased persistence in pecking at preferred food (Rogers and Anson, 1978) and abnormal social behaviour (Rogers *et al.*, 1974). The injection parameters of dose, volume and age of chicks were critical in obtaining all these effects (Rogers and Anson, 1978; Rogers *et al.*, 1978).

Hambley and Rogers (1979) have explained the mechanism whereby cycloheximide produces these behavioural effects. They do not appear to be a result of the inhibition of protein synthesis *per se*, but of changing amino acid pools as a result of the inhibition of protein synthesis. In particular, brain glutamic acid and aspartic acid were substantially elevated after injection of cycloheximide on Day 2. These authors found that intracerebral

injection of a mixture of 22 physiological amino acids produced deficits on the pebble floor performance. Further examination showed that this behavioural effect could be traced to four amino acids, GABA, taurine, glutamic acid and aspartic acid. However, since cycloheximide only raised the amounts of the latter two amino acids, it was suggested that only glutamatic acid and aspartatic acid would be involved in the production of the deficits.

The hypothesis of Hambley and Rogers (1979) is that an uncontrolled stimulation of central glutamic acid or aspartic acid receptors, resulting from the increased level of the respective amino acids, caused the 'retarded' learning effect. The fact that this effect could only be found during the first week of the chick's life was considered to be due to inefficient uptake mechanisms for these amino acids in the young brain. The central uptake mechanisms for these amino acids develop roughly in parallel with the maturation of the blood-brain barrier (Lajtha and Seshen, 1980). In support of the hypothesis, Sdraulig *et al.* (1980) demonstrated that when the sodium-dependent re-uptake mechanisms were inhibited by ouabain in older chickens, glutamic acid injections resulted in pebble floor performance deficits. In addition, Rogers and Hambley (1981) have demonstrated that the potent glutamic acid and aspartic acid agonists, kainic acid and N-methyl-aspartic acid were effective in producing 'retarded' learning. However, α-methyl-aspartic acid, which is electrophysiologically

inactive, does not produce any behavioural deficits. Kainic acid appeared to be the most potent agent for producing cycloheximide-like 'retarded' learning in chickens.

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CHAPTER TWO

THE STRIATUM AND MOTOR BEHAVIOUR

Chapter 2.

The Striatum and Motor Behaviour

This chapter consists of the following parts and appendices:

Part 2.1 Chapter overview.

Part 2.2.1 Kainic acid injections in the striatum alter the cataleptic and locomotor effects of drugs influencing dopaminergic and cholinergic systems.

Part 2.2.2 Sedative effects of apomorphine in an animal model of Huntington's disease.

Part 2.2.3 Haloperidol-induced catalepsy is mediated by post-synaptic dopamine receptors.

Part 2.2.4 Experiential influences on catalepsy.

Part 2.2.5 Dopaminergic and cholinergic influences on motor behaviour in chickens.

Part 2.2.6 The effect of striatal lesions in the chick on haloperidol-potentiated tonic immobility: a preliminary study.

Part 2.2.7 Permanent effects of injections of cycloheximide or glutamate in the chick forebrain on motor activity following apomorphine or scopolamine.

Appendix 1 Amphetamine-induced locomotor activity and stereotypy after kainic acid lesions of the striatum.

Appendix 2 Kainic acid lesions of the striatum dissociate amphetamine and apomorphine stereotypy: similarities to Huntington's chorea.

Appendix 3 Effects of lithium carbonate on haloperidol-induced catalepsy in rats.

Part 2.1 Chapter overview

The traditional role attributed to the striatum is that of the regulation of motor behaviour. Dysfunction of various aspects of the striatum can lead to classical motor diseases such as Parkinson's disease, Huntington's disease and tardive dyskinesia in humans (Yahr, 1976). The role of the striatum in motor behaviour is not clearly understood. However, animal research over the past decade has started to unravel striatal function.

In rats, the function of striatal dopamine has been extensively studied since Ungerstedt first showed that the dopaminergic nigro-striatal neurons could be lesioned selectively with 6-hydroxydopamine (Ungerstedt and Arbuthnoff, 1970). Manipulation of the nigrostriatal pathway produces easily measurable behavioural changes in rats. When this pathway is lesioned by 6-hydroxydopamine, the alterations in motor activity which ensue are highly correlated with the degree of depletion of striatal dopamine and the amount of neuronal pathology in the substantia nigra (Ungerstedt, 1971). Unilateral lesions tend to make rats spontaneously circle towards the lesioned side. However, when the release of dopamine is increased in the remaining nigro-striatal system, by administration of d-amphetamine, for example, this circling tendency is changed to continuous rotation. On the other hand, apomorphine causes the animals to circle contralaterally to the lesioned side by stimulation of dopamine receptors in the ipsilateral striatum that have become supersensitive as a result of denervation (Pycock, 1980). These dopamine receptor stimulants also produce stereotyped behaviours, including repetitive sniffing, licking and gnawing (Iversen, 1977). These techniques are constantly

in use by investigators exploring the relationship between dopaminergic systems in the striatum and motor behaviour.

The discovery that kainic acid could selectively destroy intrinsic and efferent striatal neurons, while leaving intact afferent and traversing pathways (Coyle *et al.*, 1978) allowed for the first time a model system with an intact dopaminergic nigrostriatal pathway, but loss of post-synaptic neurons. One of the major discoveries of research using this model system was the demonstration of dopamine receptors localised, not only on post-synaptic striatal neurons, but on terminals of the nigrostriatal and corticostriatal pathways as well (for review see Dray, 1979). Figure 1 illustrates the three types of dopamine receptors found in the striatum. The studies reported in the first parts of this chapter explore the effects of dopaminergic drugs on motor behaviour of rats with kainic acid-induced striatal lesions.

One of the best documented functions of the striatum is its role in mediating the motor stereotypies seen after administration of high doses of d-amphetamine (Iversen, 1977). This stereotypy-producing effect seems to depend on the integrity of the dopaminergic nigrostriatal pathway (Ungerstedt, 1971a). A related dopaminergic pathway ascending in part from the ventral tegmental area (Ungerstedt, 1971b), innervating the nucleus accumbens and other mesolimbic and mesocortical areas, has been implicated in the mediation of locomotor hyperactivity produced by lower doses of d-amphetamine (Pinjenburg *et al.*, 1976). We found that after kainic acid-induced striatal lesions, rats show enhanced responsiveness to both the locomotor and stereotypy-inducing effects

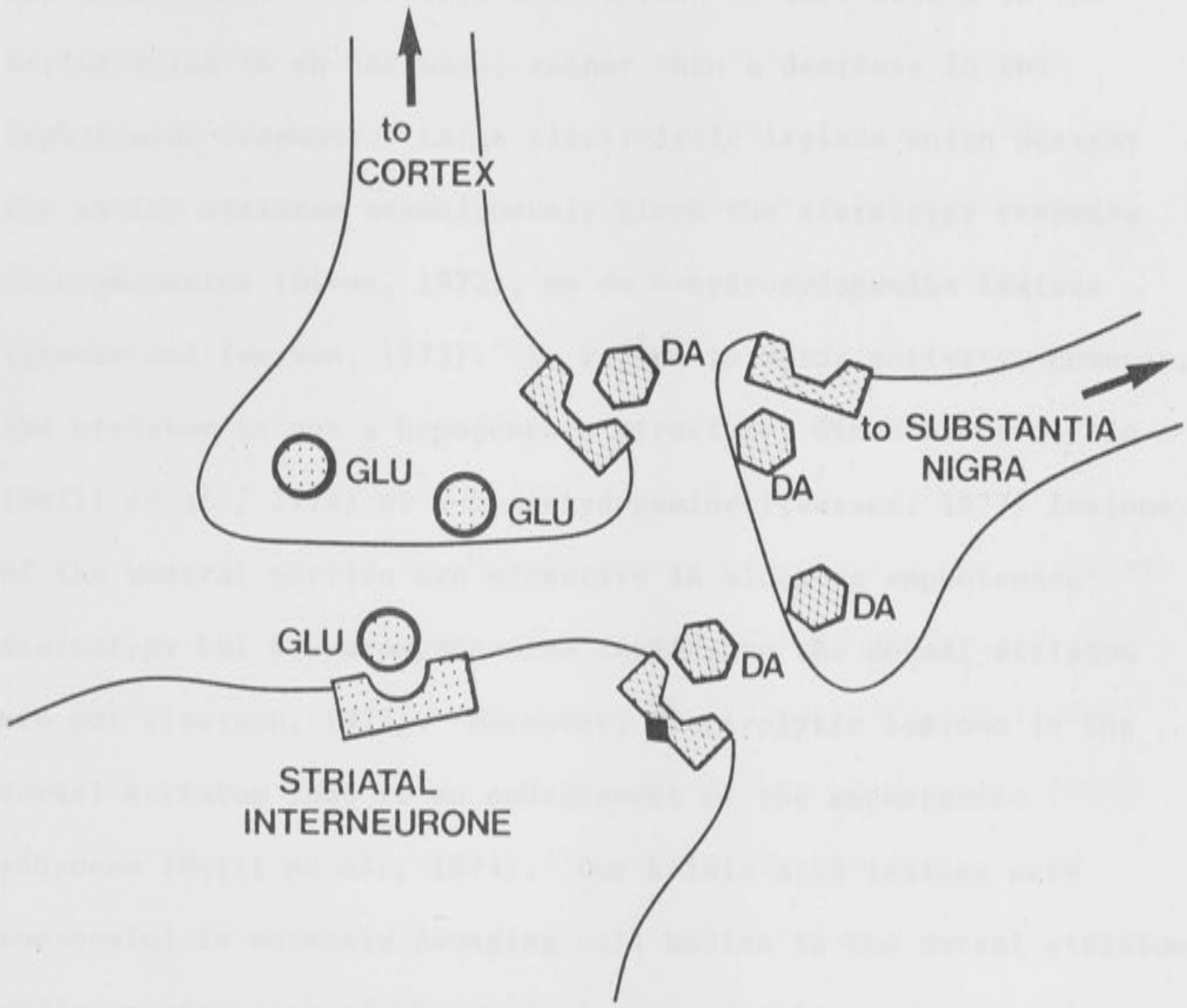

Figure 1. Pictoral illustration of striatal dopamine (DA) receptors localized on 1) striatal interneurones, 2) glutamatergic (GLU) cortico-striatal terminals and 3) dopaminergic nigro-striatal terminals.

of d-amphetamine (Appendix 1, Mason *et al.*, 1978a; Appendix 2, Mason *et al.*, 1978b; Part 2.2.1, Sanberg *et al.*, 1981). It was paradoxical that severe destruction of cell bodies in the striatum led to an increase, rather than a decrease in the amphetamine response. Large electrolytic lesions which destroy the entire striatum unambiguously block the stereotypy response to amphetamine (Divac, 1972), as do 6-hydroxydopamine lesions (Creese and Iversen, 1973). In regard to motor activity, however, the striatum is not a homogeneous structure, since electrolytic (Neill *et al.*, 1974) or 6-hydroxydopamine (Iversen, 1977) lesions of the ventral portion are effective in blocking amphetamine stereotypy but 6-hydroxydopamine lesions to the dorsal striatum are not (Iversen, 1977). Moreover, electrolytic lesions in the dorsal striatum lead to an enhancement of the amphetamine response (Neill *et al.*, 1974). Our kainic acid lesions were successful in severely damaging cell bodies in the dorsal striatum while sparing much of the ventral part of this structure, as shown by histology and regional biochemical assay. Thus, our lesions correspond more closely to the dorsal electrolytic lesions of Neill *et al.* (1974), which also showed an increase in the amphetamine response. This suggests that the motor outflow from the striatum necessary for the expression of stereotypy and damaged by global striatal lesions may be more localised to the ventral half.

It is still necessary, however, to explain why dorsal striatal lesions increase the amphetamine response rather than having no effect, as a ventrally-located motor outflow model would suggest. It has been found that kainic acid injection into the striatum abolished the usual inhibition of single

units in the substantia nigra (Rebec and Groves, 1975) in response to systemic d-amphetamine (Groves, 1979). This suggests that part of the striato-nigral feedback loop was destroyed by the kainic acid lesion (Dray, 1979) and that the effects of amphetamine may be two-fold; (1) releasing dopamine in the nucleus accumbens, producing locomotor activity (Pijnenburg *et al.*, 1976), and in the ventral striatum producing stereotypy (Iversen, 1977), and (2) activating a striato-nigral negative feedback loop which inhibits the activity of dopaminergic neurons in the substantia nigra, pars compacta. Since it is known that the amount of dopamine released onto the post-synaptic cell is a combined function of the dose of amphetamine and the firing of neurons in the substantia nigra, pars compacta (Van Voigtlander and Moore, 1973), the amount of dopamine being released onto the post-synaptic receptors in the striatum or nucleus accumbens would be controlled by a balance of these two, opposing actions of amphetamine. Therefore, if a substantial portion of the striato-nigral feedback loop originates in the dorsal striatum, while the primary motor output region is located in the nucleus accumbens and ventral striatum, then a selective lesion of the dorsal striatum would result in enhanced release of dopamine in nucleus accumbens and ventral striatum, and hence in increased motor effects of amphetamine. This model assumes that the mesolimbic projection to the nucleus accumbens is also under feedback control from the striatum. In this regard, it is of interest that dopamine projections to the nucleus accumbens do indeed originate in part from medial substantia nigra, pars compacta (A9) cells (Dahlstrom and Fuxe, 1964), not exclusively from the A10 area (Carter and Fibiger, 1977) of the brainstem.

In another study (Appendix 2, Mason *et al.*, 1978b) we found that apomorphine-induced stereotypy was not enhanced in rats with striatal lesions induced by kainic acid. Apomorphine, since it acts directly on the postsynaptic dopamine receptor (Anden *et al.*, 1967) would have effects independent of the release of dopamine from the presynaptic terminal, and so would not be affected one way or the other by kainic acid lesions of the dorsal striatum. Thus, disruption of a striato-nigral inhibitory feedback loop originating in the dorsal striatum may be capable of explaining both the paradoxical potentiation of amphetamine locomotor activity and stereotypy, and the lack of effect on apomorphine stereotypy in kainic acid-lesioned rats.

In contrast to the effects of high doses of apomorphine in enhancing activity, Maj *et al.* (1977) demonstrated that low doses of apomorphine have a sedative action on rats. The effect of low doses is presumably mediated by stimulation of presynaptic dopamine receptors localized on terminals of the dopaminergic nigrostriatal tract, i.e., autoreceptors (Corsini *et al.*, 1978). Since rats with kainic acid-induced striatal lesions still have the nigrostriatal tract intact, with normal levels of dopamine and tyrosine hydroxylase (Coyle *et al.*, 1978), we were interested in observing the sedative effects of apomorphine in these animals. In Part 2.2.2 (Sanberg *et al.*, 1979) we found that while the locomotor effects of amphetamine were enhanced as discussed above, the rats did not respond any differently from controls while under low doses of apomorphine. This suggests that striatal dopamine autoreceptors, responsible for motor activity inhibition,

function normally in kainic acid-lesioned rats.

Dopamine receptor blockers (i.e., neuroleptics) produce a behavioural state in animals in which they fail to correct externally imposed postures, referred to as catalepsy. Although there is increasing evidence that locomotor activity is influenced by the mesolimbic dopamine system (as discussed above), neuroleptic-induced catalepsy appears to be more dependent on the nigro-striatal dopamine system. This is based on the finding that electrolytic lesions of the striatum, but not electrolytic lesions of the nucleus accumbens, greatly reduce catalepsy induced by neuroleptics (Costall and Olley, 1978; Fog *et al.*, 1970; Honma and Fukushima, 1978; Koffer *et al.*, 1978). Within the striatum, neuroleptics such as haloperidol act at dopamine receptor sites (Creese *et al.*, 1975, 1976). Dopamine receptors, identified by the specific, high affinity binding of haloperidol, have been shown to be equally distributed postsynaptically on striatal neurones and presynaptically on corticostriatal terminals (Schwarcz *et al.*, 1978). Because electrolytic lesioning unavoidably damages both pre- and postsynaptic striatal dopamine receptors, it was not known whether these two receptors were separately involved in neuroleptic-induced catalepsy. To answer this question, we used kainic acid (Part 2.2.1, Sanberg *et al.*, 1981; Part 2.2.3, Sanberg, 1980) and cortical ablations (Sanberg, 1980) to destroy postsynaptic and presynaptic dopamine receptors, respectively. Haloperidol-induced catalepsy was found to be greatly reduced in rats with striatal lesions induced by kainic acid, whereas decorticate rats did not show this reduction. The results demonstrate that the cataleptic effects of haloperidol are mediated by dopamine receptors localised postsynaptically on striatal neurons.

A possible role in motor behaviour for the presynaptic dopamine receptors localized on the terminals of the cortico-striatal tract has not yet been proposed. A possible clue to their function was found in a preliminary study (Appendix 3, Faulks and Sanberg, 1980). It was found that cortical lesions attenuate the potentiating effect of lithium carbonate on haloperidol-induced catalepsy. Removal of the frontal and parietal cortices partially eliminates the dopamine receptors localised on cortico-striatal fibres. Thus, it is possible that lithium acts in some way on these receptors to potentiate the haloperidol cataleptic effects. Further work is needed to clarify these findings.

The striatum contains a large amount of acetylcholine, which is considered the major transmitter of intrinsic neurons in the striatum (Coyle *et al.*, 1978). Kainic acid injections into the striatum markedly reduced the number of cholinergic neurons (Coyle *et al.*, 1978). It was therefore of interest to look at the effects of cholinergic drugs on the motor behaviour of rats with these lesions. In normal motor behaviour there appears to be a balance between cholinergic and dopaminergic mechanisms within the striatum (Barbeau, 1973). While dopamine agonists and antagonists increase and decrease motor activity, respectively, injections of cholinergic agonists or antagonists cause decreases or increases in motor activity, respectively (Barbeau, 1973). In Part 2.2.1 (Sanberg *et al.*, 1981) we found that the cataleptic effects of the cholinergic agonist, pilocarpine, and the locomotor-stimulating effects of the cholinergic antagonist, scopolamine, were significantly enhanced in rats with kainic acid-induced lesions of the striatum.

The presumed hyper-responsive mesolimbic dopamine system discussed above in regard to the effects of amphetamine, may also be involved in the enhanced locomotor response to scopolamine. Watanabe *et al.* (1978) demonstrated that scopolamine-induced locomotor activity is dependent upon intact dopamine systems. Costall *et al.* (1979) showed an antagonistic relationship between acetylcholine and dopamine within the nucleus accumbens with respect to locomotor activity. Stephens and Herberg (1979) found that scopolamine injected into the nucleus accumbens was especially effective in blocking neuroleptic antagonism of hypothalamic self-stimulation, and Snyder (1976) has suggested that anticholinergic agents might counteract the action of neuroleptic drugs by inhibiting the reuptake of dopamine, thus increasing its availability at receptor sites.

The potentiation of pilocarpine-induced catalepsy in rats with kainic acid lesions of the striatum is similar to that observed by Costall and Olley (1971) after administration of arecoline in rats with electrolytic lesions of the striatum. Also, Silbergeld and Hruska (1979) showed that rats with kainic acid-induced striatal neurodegeneration are especially sensitive to the tremorogenic, rotational and seizure-inducing effects of arecoline and tremorine. Kainic acid injections in the striatum reduce the number of striatal muscarinic receptors without changing the affinity of the remaining binding sites (Hruska *et al.*, 1978). Silbergeld and Hruska (1979) therefore suggested that the increased responses to cholinergic agonists may be due to an increase in the sensitivity of cholinergic receptors in non-striatal areas as a result of nonspecific damage from the kainic acid injection. Further work is needed to verify

this interpretation. Nevertheless the results of all the studies demonstrate profound influences on motor behaviour in rats following removal of striatal neurons with kainic acid. This lesioning technique has allowed us to study the role of specific neurotransmitter systems in controlling motor behaviour in ways not previously available.

The Avian Striatum and Motor Behaviour

Compared to what is known about motor behaviour in mammals, the role of dopaminergic and cholinergic systems in motor behaviour in birds is little known. Stereotyped behaviours in birds, such as repetitive head-neck movements, compulsive preening, pecking and vocalisation are induced by injections of the dopamine agonist, apomorphine (Nistico' and Stephenson, 1979), and seem to be mediated by the dopaminergic tegmentopaleostriatal system, the homologue of the dopaminergic nigrostriatal system in mammals (Goodman and Azzaro, 1978; Nistico' and Stephenson, 1979). While most studies have been performed with pigeons (Nistico' and Stephenson, 1979) chickens also exhibit similar stereotyped behaviours following apomorphine injections (de Lanerolle and Youngren, 1978; Machlis, 1980; Osuide and Adejoh, 1973). As in mammals, chickens also show increased locomotor activity in response to the dopaminergic agonists, d-amphetamine (Spooner and Winters, 1966; Wallach *et al.*, 1972) and L-dopa (Wallach *et al.*, 1972). In all these experiments with chickens the increase in motor behaviour was recorded by methods of direct observation. Thus, time course studies have been limited. Recently, Wallnau *et al.* (1979) measured activity increases on automatic

stabilimeters after one dose of apomorphine for 10min. On the other hand, Wallnau *et al.* (1979) demonstrated that the dopamine receptor blocker, haloperidol, prolonged tonic immobility in chickens. In Part 2.2.4 (Sanberg *et al.*, 1980) we found catalepsy as measured in rodents to be similar to the tonic immobility behaviour measured in chickens. Thus haloperidol-potentiated tonic immobility in chickens may be an analogue of haloperidol-induced catalepsy in rats.

The effect of cholinergic agents on motor behaviour in birds are less known than the effects of dopaminergic drugs. As mentioned above, anticholinergics such as scopolamine increase locomotor activity in rats. Two available studies which have been performed with chickens have shown enhanced activity, as measured in photocell activity cages, following scopolamine administration (Thompson *et al.*, 1974; Ksir, 1978). The reported effects of scopolamine on tonic immobility, however, have been contradictory. Thompson *et al.* (1974) and Woodruff *et al.* (1976) have shown decreased tonic immobility following injections of scopolamine in chickens and ducks, respectively. Ksir (1978) on the other hand, found a lack of effect of scopolamine on tonic immobility in chickens.

Because of the small amount of knowledge and contradictory information on the influence of cholinergic and dopaminergic systems on motor behaviour in birds, the studies reported in Part 2.2.5 were conducted to clarify further the role of the transmitter systems in motor behaviour in chickens. The apparatus designed and used for measuring motor activity in chickens are shown in Figures 2 and 3. Figure 4 shows a chicken in tonic immobility.

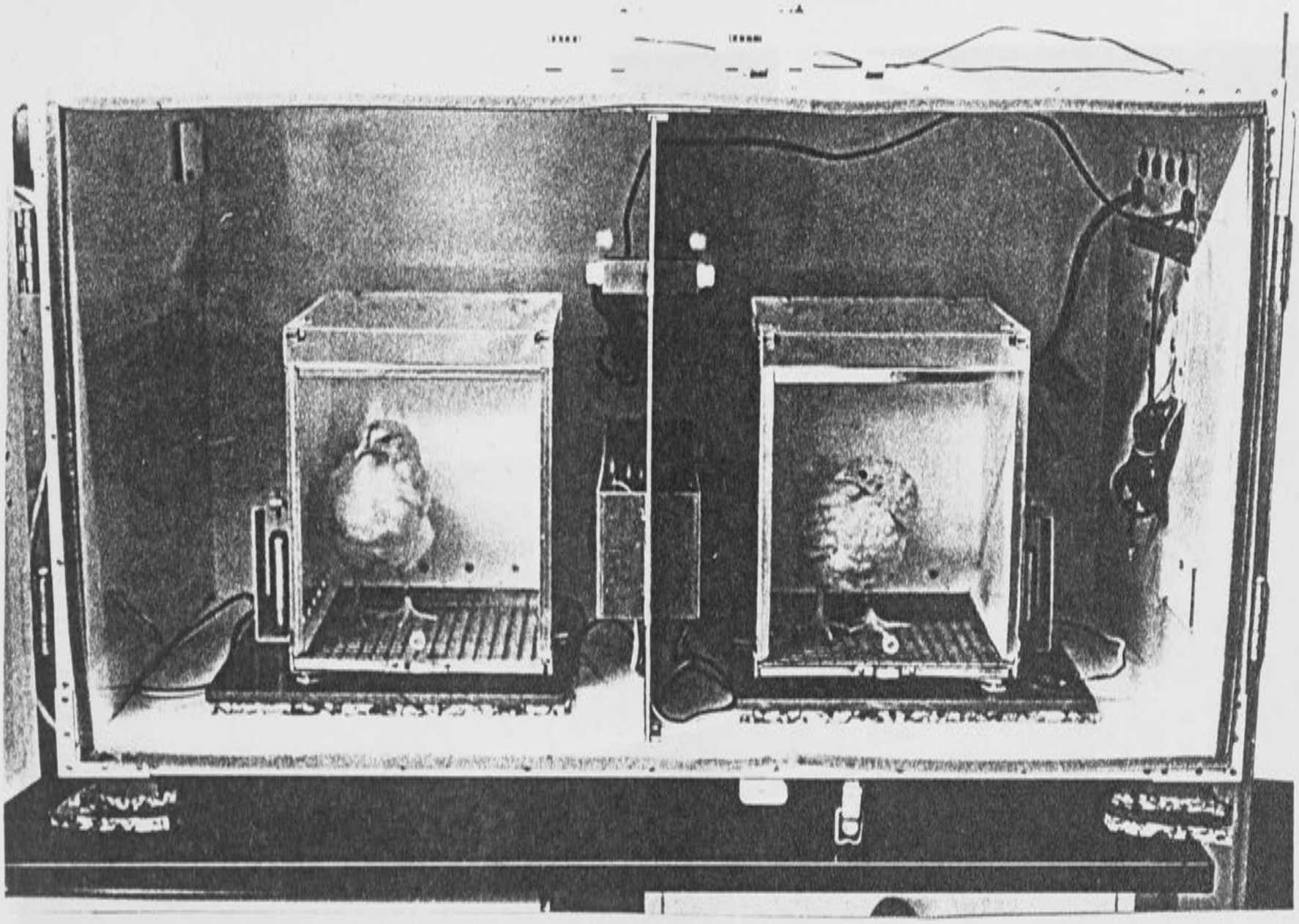

Figure 2. Photograph of chickens in activity cages located in separate chambers of a large BRS sound-proof chamber. During testing the doors are closed.

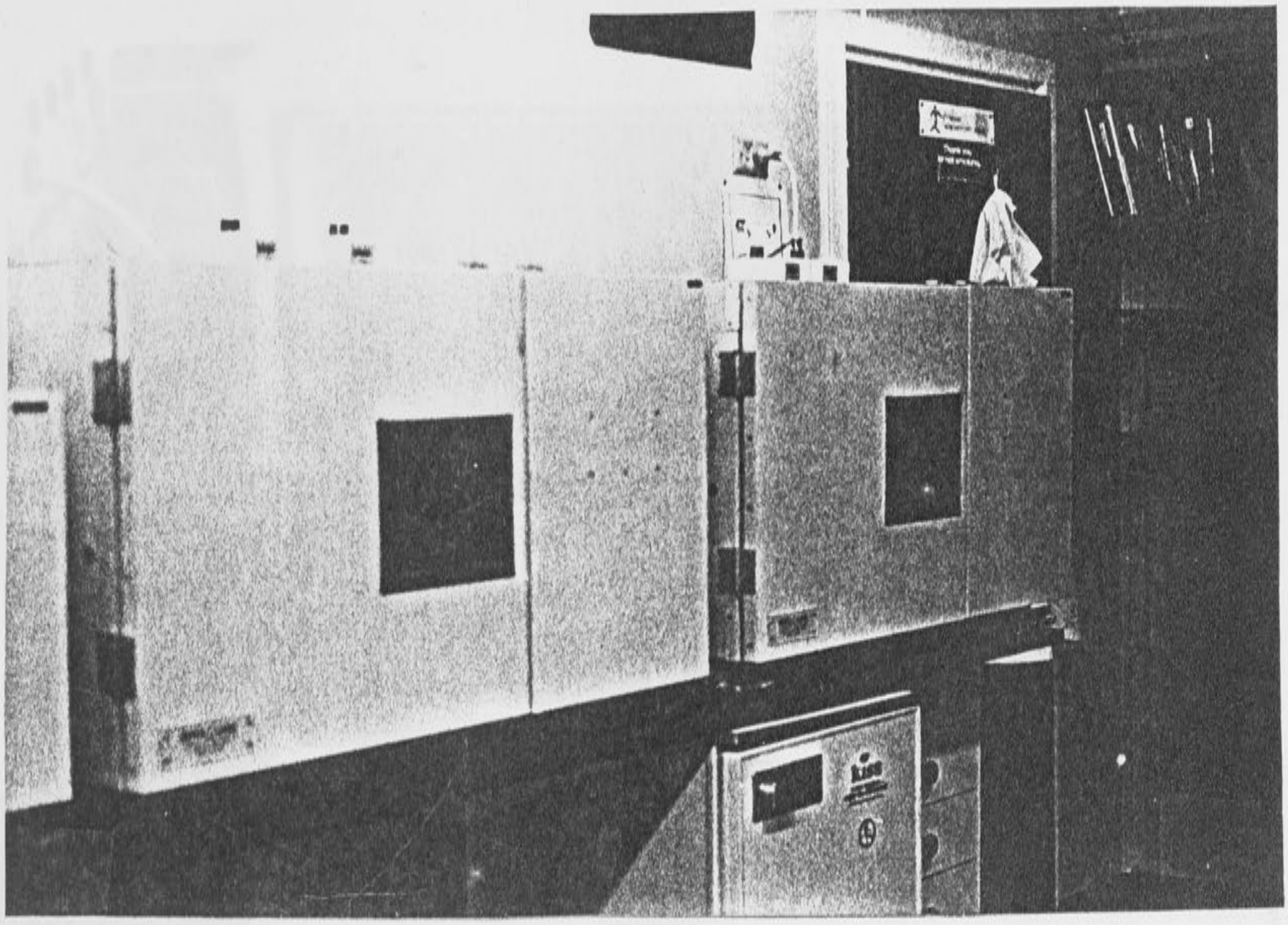

Figure 3. Photograph of laboratory showing two large BRS sound-proof chambers.

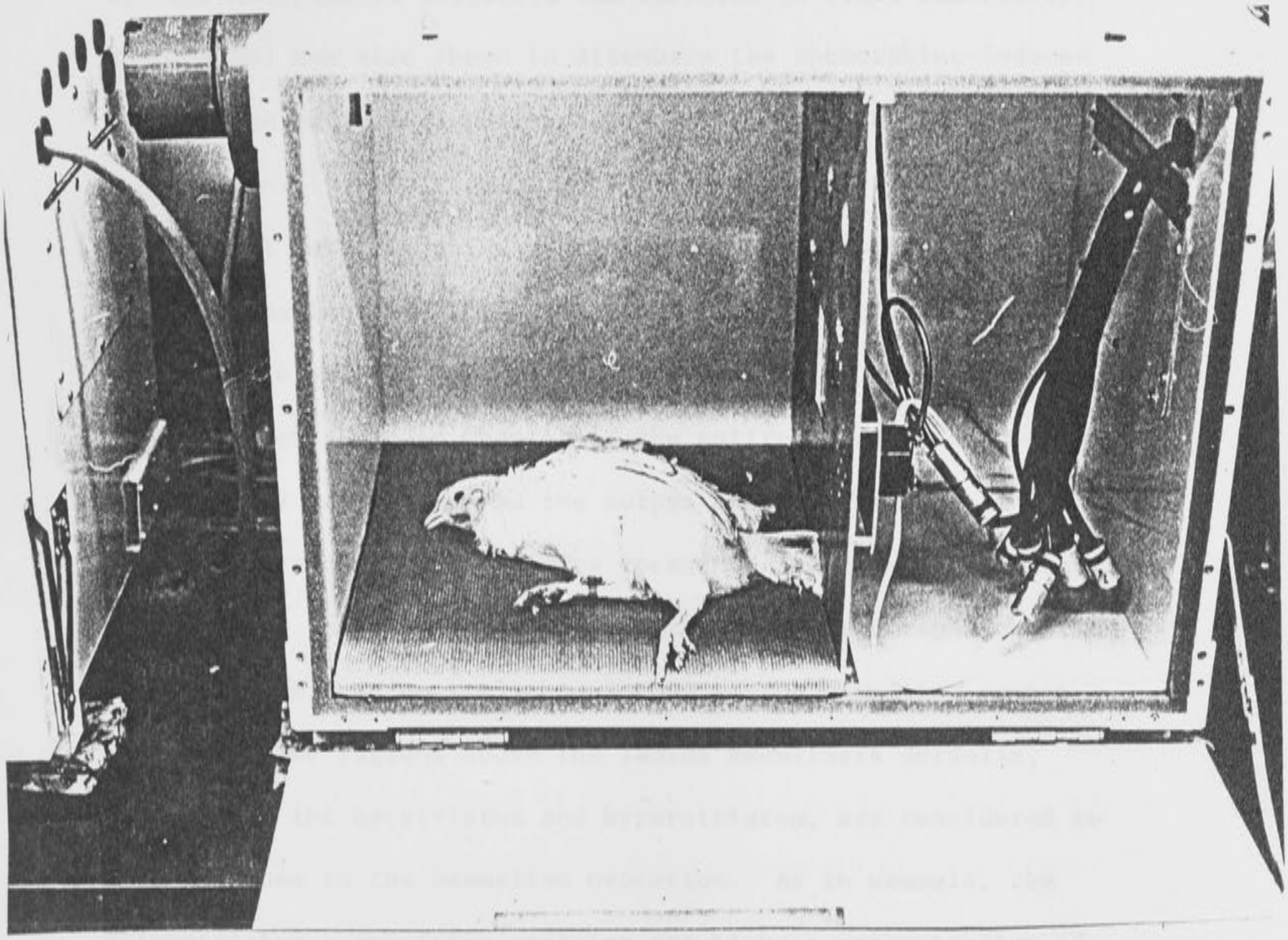

Figure 4. Photograph of chicken in a typical tonic immobility position. Testing was done in a small BRS sound-proof chamber as shown.

In agreement with previous studies, it was found that apomorphine enhanced stabilimeter motor activity in chickens, whereas haloperidol increased the duration of tonic immobility. Haloperidol was also shown to attenuate the apomorphine-induced increase in activity. Motor activity could also be increased by scopolamine. In addition, scopolamine was shown to decrease the duration of tonic immobility. These results support the involvement of dopaminergic and cholinergic systems in controlling motor behaviour in birds.

As mentioned in Chapter 1, the entire avian telencephalon was commonly referred to as the corpus striatum because of its gross appearance. However, more recent anatomical and histochemical studies have found only the basal part of the telencephalon, the paleostriatum, may be truly homologous to the mammalian corpus striatum. The regions above the lamina medullaris dorsalis, principally the neostriatum and hyperstriatum, are considered to be homologous to the mammalian neocortex. As in mammals, the paleostriatum augmentatum contains the highest concentration of dopamine, which probably originates via the tegmentopaleostriatal tract (Nistico' and Stephenson, 1979). The paleostriatum augmentatum also receives a topographical projection from the neostriatum, which is considered to be homologous to the mammalian corticostriatal pathway (Karten, 1969; Karten and Dubbeldam, 1973). In rats, it was found that destruction of the cortex and the dopamine receptors localised on the corticostriatal terminals had no effect on haloperidol-induced catalepsy (Part 2.2.3, Sanberg *et al.*, 1980). However, lesions of intrinsic striatal neurons and their respective postsynaptic dopamine receptors by kainic acid

injections attenuated haloperidol's effects. Since there has been little work done showing a functional homology between the avian neostriatum-paleostriatum, and the mammalian neocortex-basal ganglia, we thought it of interest to determine the effects of lesions of the neostriatal and paleostriatal regions of the chick telencephalon on haloperidol-potentiated tonic immobility in chickens.

In Part 2.2.6, neo- and hyperstriatal lesions were made in chicks with aspiration to the level of the lamina medullaris dorsalis as shown in the hatch marks of Fig. 5. Paleostriatal lesions were produced by injections of kainic acid into the paleostriatum complex, as described by Rieke (1980).

It was found that removal of the neostriatal and hyperstriatal areas had no effect on haloperidol's ability to potentiate tonic immobility. However, kainic acid injections into the paleostriatum decreased the effectiveness of haloperidol's action. This is similar to that seen in rats which received decortication or injections of kainic acid into the striatum. Thus, these behavioural results support the premise, originally based on anatomical and histochemical findings (Karten and Dubbeldam, 1973), that the neostriatum and hyperstriatum in birds is homologous to the neocortex of mammals, whereas the avian paleostriatum is the homologue of the mammalian corpus striatum, at least as far as motor behaviour is concerned.

These findings (Part 2.2.6) that bilateral paleostriatal lesions reduced the effects of the dopamine receptor antagonist, haloperidol, extend the work of Goodman and Stitzel (1977). They showed that the effects of the dopamine receptor agonist, apomorphine, are decreased in pigeons given electrolytic lesions

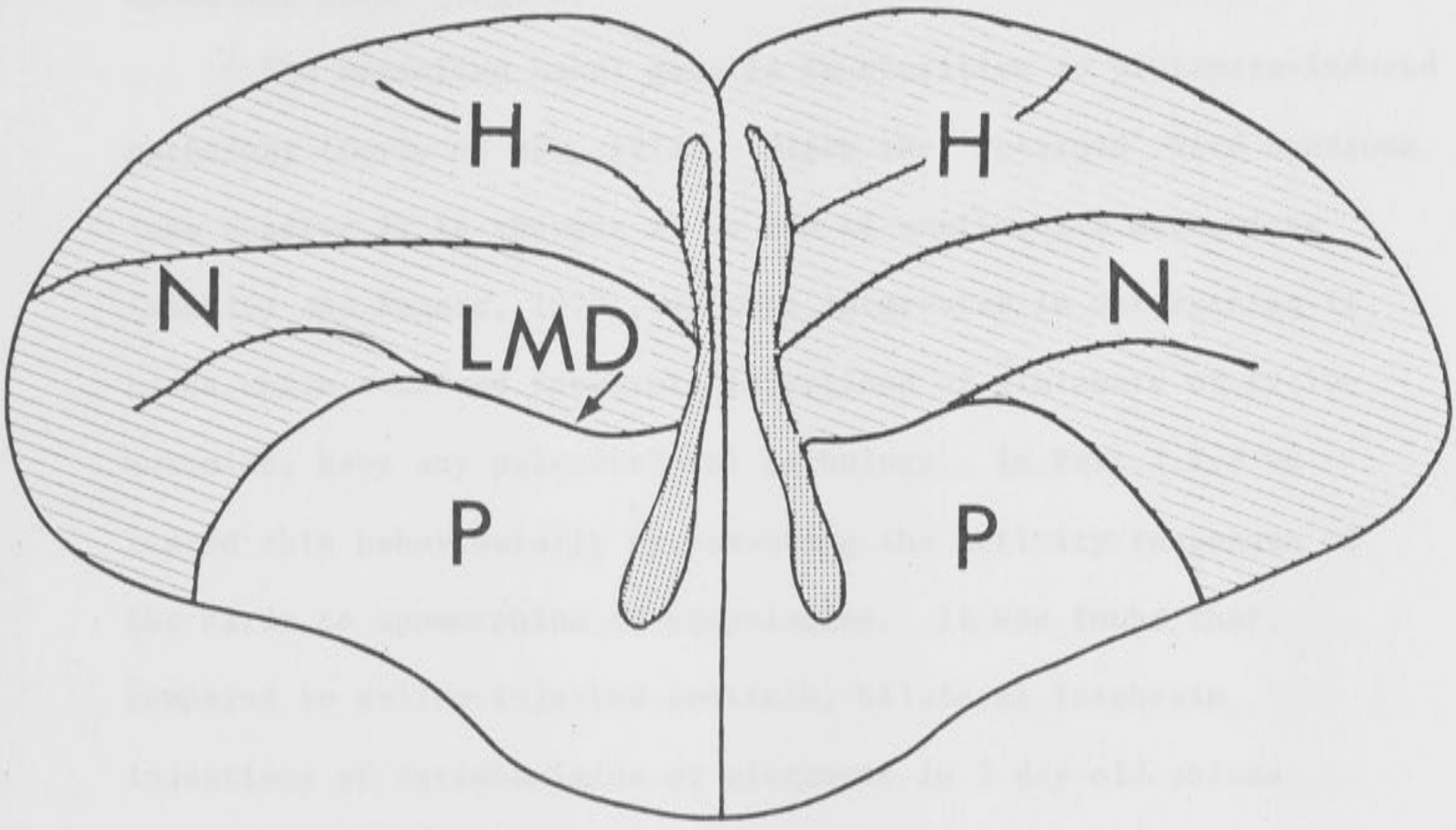

Figure 5. Schematic representation of coronal section of chick forebrain. Hatched marks show the areas removed in partially decerebrated chicks. H, hyperstriatum; N, neostriatum; P, paleostriatum; LMD, lamina medullaris dorsalis.

of the paleostriatum. Continued research, determining the nature of dopamine receptor mechanisms in the avian brain, may prove useful in proving if the avian paleostriatum is truly homologous to the mammalian basal ganglia.

The mammalian basal ganglia is sensitive to glutamate-induced pathology (Coyle *et al.*, 1978). Since the 'retarded' bird syndrome (see Chapter 1) is thought to be due to excitotoxic mechanisms (Hambley and Rogers, 1979), we were interested in determining if birds which received forebrain injections of glutamate or cycloheximide, have any paleostriatal pathology. In Part 2.2.7 we tested this behaviourally by measuring the activity responses of the birds to apomorphine or scopolamine. It was found that, compared to saline-injected controls, bilateral forebrain injections of cycloheximide or glutamate in 2 day-old chicks resulted in significantly less activity in response to intraperitoneal injections of the dopaminergic agonist, apomorphine, or the cholinergic antagonist, scopolamine, when tested on stabilimeters (see Fig. 3) four weeks later.

If the striatum and the avian paleostriatum are truly homologous (as discussed above) there are two likely anatomical interpretations of the results. The first is that cholinergic interneurons are destroyed by the glutamate or cycloheximide injections. This would result in less postsynaptic dopamine receptors through which apomorphine could exert its effects and in denervated cholinergic receptors on efferent neurons. If these muscarinic cholinergic receptors became supersensitive, then the effects of the anticholinergic, scopolamine, would be

decreased, compared to controls. The other explanation is that the efferent neurons or both efferent and intrinsic neurons in the striatum are decreased, thus reducing any output from this structure induced by dopaminergic or cholinergic mechanisms. The latter explanation seems more plausible, since the former requires that these gross injections of relatively large amounts of glutamate or cycloheximide are more specific than localized intrastriatal injections of glutamate, which destroy both intrinsic and efferent neurons (Coyle *et al.*, 1978).

In birds, these anatomical interpretations are only speculative until further work on the homology between basal ganglia areas of birds and mammals is done, moreover, a positive identification of paleostriatal neuronal destruction in cyclo-heximide and glutamate-treated birds is essential. Nevertheless, the results suggest that cycloheximide and glutamate injections are causing permanent alterations in dopaminergic and cholinergic neurotransmitter mechanisms, which may be associated with paleostriatal neurons. Changes in these transmitter systems may explain some of the behavioural abnormalities previously described (Hambley and Rogers, 1979; Rogers and Anson, 1978; Rogers *et al.*, 1974; Sanberg *et al.*, 1981; Sdraulig *et al.*, 1980 and Chapter 1).

My present research is concerned with outlining further the effects of dopaminergic and cholinergic drugs on motor behaviour in chickens (Part 2.2.5). Specifically, the effects of a cholinergic agonist (tremorine) on motor activity and tonic immobility are being investigated. In the chicken studies, where various manipulations to the telencephalon were given

(Parts 2.2.6 and 2.2.7), detailed histological and biochemical analyses are being carried out. This will allow better association of the behavioural results with neurological mechanisms, and provide more evidence for functional, biochemical and anatomical homologies of the paleostriatum and neostriatum in birds to the basal ganglia and neocortex, respectively, in mammals.

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KAINIC ACID INJECTIONS IN THE STRIATUM ALTER THE CATALEPTIC AND LOCOMOTOR EFFECTS OF DRUGS INFLUENCING DOPAMINERGIC AND CHOLINERGIC SYSTEMS.

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Submitted for publication to

European Journal of Pharmacology

ABSTRACT

Rats with bilateral injections of kainic acid into the striatum were tested for their motor responsiveness to drugs influencing dopaminergic or cholinergic systems. The kainic acid induced lesions potentiated the locomotor response to both the dopaminergic agonist, d-amphetamine, and the cholinergic antagonist, scopolamine, attenuated the cataleptic response to the dopamine antagonist, haloperidol, and potentiated the cataleptic and convulsive responses to the cholinergic agonist, pilocarpine. The analogy of these pharmacological effects with those induced by similar drugs in patients with Huntington's disease supports the view that this animal preparation is a useful model of Huntington's disease. The opposite effects of haloperidol and pilocarpine on catalepsy in kainic acid lesioned rats suggest that more work should be done to elucidate the mechanism behind this conflict before full support can be given to its use as a model system for evaluating possible pharmacotherapy in HD.

Key words: Striatum. Kainic acid. Haloperidol. d-amphetamine. Scopolamine. Pilocarpine. Locomotor activity. Catalepsy. Huntington's disease.

1. INTRODUCTION

Several studies have recently indicated morphological, biochemical and behavioural similarities between the effects of striatal injections of kainic acid (KA) in rats and those of Huntington's disease (HD) in man. Thus, KA injections destroy the neuronal perikaria of the striatum, with an associated marked decrease of cholinergic and GABAergic neurochemical markers, and no apparent effect on either the dopaminergic nigro-striatal fibers or the glutamatergic cortico-striatal fibers (see Coyle *et al.*, 1978 for a review). These morphological and biochemical effects are similar to those found in HD patients (Barbeau, 1973; Bruyn, 1968; De Silva, 1977; Klawans and Weiner, 1976; McGeer and McGeer, 1976). Furthermore, behavioral alterations interpreted to be analogous to the chorea and dementia of HD have been reported in rats after KA injections in the striatum (Divac *et al.*, 1979; Hruska and Silbergeld, 1979; Pisa *et al.*, 1979, 1980a, 1980b; Sanberg *et al.*, 1978a, 1978b).

Cholinergic agonists and dopaminergic antagonists appear to attenuate and the opposite pharmacotherapy usually worsens, the chorea of HD patients (Barbeau, 1973; De Silva, 1977; Klawans and Weiner, 1976).

Previously an exaggerated motor response to administration of d-amphetamine was shown in rats with KA-induced striatal lesions (Mason *et al.*, 1978a, b; Pisa *et al.*, 1980a, Sanberg *et al.*, 1979a, b). The aim of the present study was to extend these pharmacological observations to verify further the viability of KA-induced striatal lesions as a model for pharmacotherapy of HD. Specifically, we examined the responsiveness of rats with

these lesions to the cataleptogenic effects of pilocarpine, a cholinergic agonist (Goodman and Gilman, 1955), and haloperidol, a dopamine receptor blocker (De Silva, 1977) and their responsiveness to the locomotor effects of the anti-cholinergic, scopolamine (Goodman and Gilman, 1955) and the dopamine agonist, d-amphetamine (Mason *et al.*, 1978a, b). A preliminary report has been presented elsewhere (Sanberg *et al.*, 1979c).

2. MATERIALS AND METHODS

2.1 *Subjects*

Twenty-two male Wistar rats (Woodlyn Farms, Guelph, Ontario) were used. They were housed in groups of five or six before surgery and in individual cages afterwards, with free access to food (Purina Rat Chow) and water. The colony room had a temperature of 22 - 25°C, humidity of 45 - 55% and a 12h light-dark cycle.

2.2 *Surgery*

The rats were randomly assigned to either a control (n = 10) or a kainic acid lesioned (KAL, n = 12) group (mean weights, 308g ± 12.8 (SD) and 313.5g ± 14.7, respectively). The rats were anaesthetized with sodium pentobarbital (50mg/kg i.p.) and positioned on a Kopf Stereotaxic apparatus, with the incisor bar adjusted at 4.2mm below the interaural line. Two holes were drilled in the skull and 3nmoles of KA were injected into each striatum over a 3min period through a 32 gauge Hamilton syringe at the following coordinates: 9.6mm rostral and 4.5mm dorsal to the interaural line, 2.8mm lateral to the sagittal suture. In pilot experiments these

coordinates were shown to correspond approximately to AP = 8.4, DV = 0.8, and ML = 2.2 of the König and Klippel atlas (1963). After the injection, the cannula was left in place for a further 5min to prevent upward movement of the solution to the neocortex along the cannula tract. The control rats were injected with the vehicle solution only.

Post-operatively, the KAL rats showed temporary stereotypy, aphagia, adipsia, little or no grooming and loss of body weight. One KAL rat died within 24h of surgery. Three other KAL rats began to bleed from their urinary tract 36h after surgery. Bleeding lasted about 24h in two rats, and about a week in the third rat, who then died of internal haemorrhage, leaving 10 rats in the KAL group. Recovery of the surviving KAL rats was promoted by forced feeding with 15ml of Soyalac® once daily, until they resumed spontaneous feeding and drinking. Also, wet mash with added Soyalac® was continuously available to the rats for about the first week post-operatively, at which time they resumed feeding on Purina Chow pellets. The aphagia and adipsia lasted about 2 - 7 days which was longer than in other studies using similar injections of KA (Sanberg and Fibiger, 1980). At the onset of the pharmacological experiments, five weeks after surgery, body weights were 430.1g ± 32.2 (SD) for controls and 367.3g ± 48.1 for KAL rats.

2.3 *Apparatus*

Catalepsy was measured using a horizontal bar (2.5cm in diameter) positioned 7.5cm above the floor. Locomotor activity was measured in six photoactometer cages (BRS Foringer No. PAC-001). They were 61cm in diameter with black walls, 43cm high, black top and grid floors. The interior

of the cages was crossed by six infrared photocell beams, each interruption of which advanced electro-mechanical counters. An automatic printout counter (BRS Foringer No. POS-112) recorded counts cumulated over periods of 10min.

2.4 *Drugs*

Three nmols of KA (Sigma Chemical Company) was dissolved in 0.5µl of a phosphate buffered isotonic saline solution (pH 7.2). Haloperidol (McNeill Laboratories), methyl scopolamine bromide (Upjohn), dextro-amphetamine sulphate (Smith, Kline and French), scopolamine hydrochloride (Sigma Chemical Company) and pilocarpine (Sigma Chemical Company) were dissolved in isotonic saline. Fresh drug solutions were prepared on each test day and injected intraperitoneally in a volume of 1ml/kg.

2.5 Procedure

2.5.1 *Catalepsy*

The rats were gently placed with their forepaws on the bar, and catalepsy measured as the time until the animal placed both paws on the floor. On each session, this procedure was repeated 60, 120, 180 and 240min after administration of the drug solution, with the animals being kept in their home cages between tests. On each test, if the rat remained on the bar for more than 5min, it was removed and assigned a score of 300sec. In the first two daily sessions the rats were adapted to the catalepsy procedure after administration of saline solution. On the third session, animals of both groups selected at random received either 1ml saline or haloperidol, in doses of 0.5, 1.0 or 2.0mg/kg. In each of the subsequent three sessions, at intervals of three days,

the rats were administered the drug solutions that they had not previously received, in a nonsystematic order.

Two weeks later the effect of pilocarpine on catalepsy was examined. Thirty minutes prior to pilocarpine administration each rat received 1mg/kg methyl-scopolamine, (a non-cataleptic agent) to block the peripheral side effects of pilocarpine, which can interfere with measurements of its centrally mediated cataleptic effects (Baez *et al.*, 1976). In the first session three rats in each group received 25mg/kg pilocarpine, four received 50mg/kg pilocarpine and three received 100mg/kg pilocarpine one hour before the first test. Since 50 and 100mg/kg pilocarpine induced intense seizure activity in KAL rats, these doses were no longer used. In the second session, one week after the first, 25mg/kg pilocarpine was administered one hour before the test to all rats that had not received this dose before. Data of only nine animals in the KAL group are reported since one KAL rat died during pilocarpine-induced serial convulsions.

2.5.2 *Locomotor Activity*

Two weeks intervened before locomotor activity was tested. One control rat selected at random was discarded, leaving nine animals in each group. Three controls and three KAL rats were each placed in individual photoactometers sometime between 10am and 12pm and their baseline activity was recorded for one hour. Three rats of each surgical group were then administered i.p. injections of either saline, 1mg/kg scopolamine or 1mg/kg d-amphetamine, respectively, and immediately replaced in their photoactometer cages for two hours. Over the next two days the remaining two groups of six rats were each treated and tested in a similar manner. All rats were then re-tested

twice, with two days of rest between sessions, each time under the influence of a drug solution that they had not previously received, using a nonsystematic order of drug administration. Each animal was tested in the same photoactometer cage in all sessions.

The experiments were run blind insofar as the experimenter did not know the identity of the drug solutions until after completion of the experiments.

2.5.3 *Statistical Analysis*

A three-way analysis of variance was conducted on the descent latencies in the catalepsy experiment and the photocell counts in the locomotor activity experiment, with Lesion as between-subject factor, and with Time and either Dose (catalepsy experiment) or Drug (locomotor activity experiment) as within-subjects factors. Tukey's tests were used to determine the statistical significance ($p < .05$) of differences among individual cell means. The data of pilocarpine-induced catalepsy were analysed with the two-tailed Student's t test.

2.5.4 *Histology*

After completion of the behavioural experiments, the rats were deeply anaesthetised with sodium pentobarbital and perfused intracardially with isotonic saline solution followed by 4% formaldehyde in saline solution. The brains were extracted and stored in 4% formaldehyde solution until sectioned. Frontal sections were cut from frozen tissue at 50μ and alternatively stained with either cresyl violet or with the Klüver and Barrera (1953) method for identification

of both cell bodies and myelinated fiber bundles.

3. RESULTS

3.1 *Histology*

The forebrain ventricles of all KAL rats were markedly dilated and both striata severely shrunken. The striatal lesions (Figure 1) were usually ovoidal, with centre approximately at A 7.8 - 8.2 and rostrocaudal diameter of 2.0 - 2.5mm. The core of both the dorsal and the ventral striatum showed complete loss of neuronal cell bodies, and marked astrocytic proliferation, with sparing of the myelinated fiber bundles of the internal capsule. The dorsomedial and ventrolateral parts of the striatum were usually unaffected. The rostral pole of the globus pallidus revealed a partial bilateral neuronal loss, astrocytic proliferation and shrinkage in all but one KAL rat, which only showed unilateral pallidal damage.

In addition to the striatal lesions, a slight loss of neurons in the juxtacallosal layers of the frontal cortex above the injection site was evident in all KAL rats. Furthermore, patches of neuronal loss were detected in the subamygdalar extent of the pyriform cortex in two KAL rats, bilaterally and in three KAL rats unilaterally (see Figure 2). The hippocampus showed morphological alterations in all but one KAL rat (see Figure 2). These alterations consisted of unilateral or bilateral patches of neuronal loss in the CA1 field, the CA3 field, or both. In one KAL rat, only shrinkage and dark-staining of the pyramidal neurons was evident in these hippocampal fields, with no cell loss.

The brains of the control rats showed a slight glial infiltration along the cannula track, otherwise they were intact.

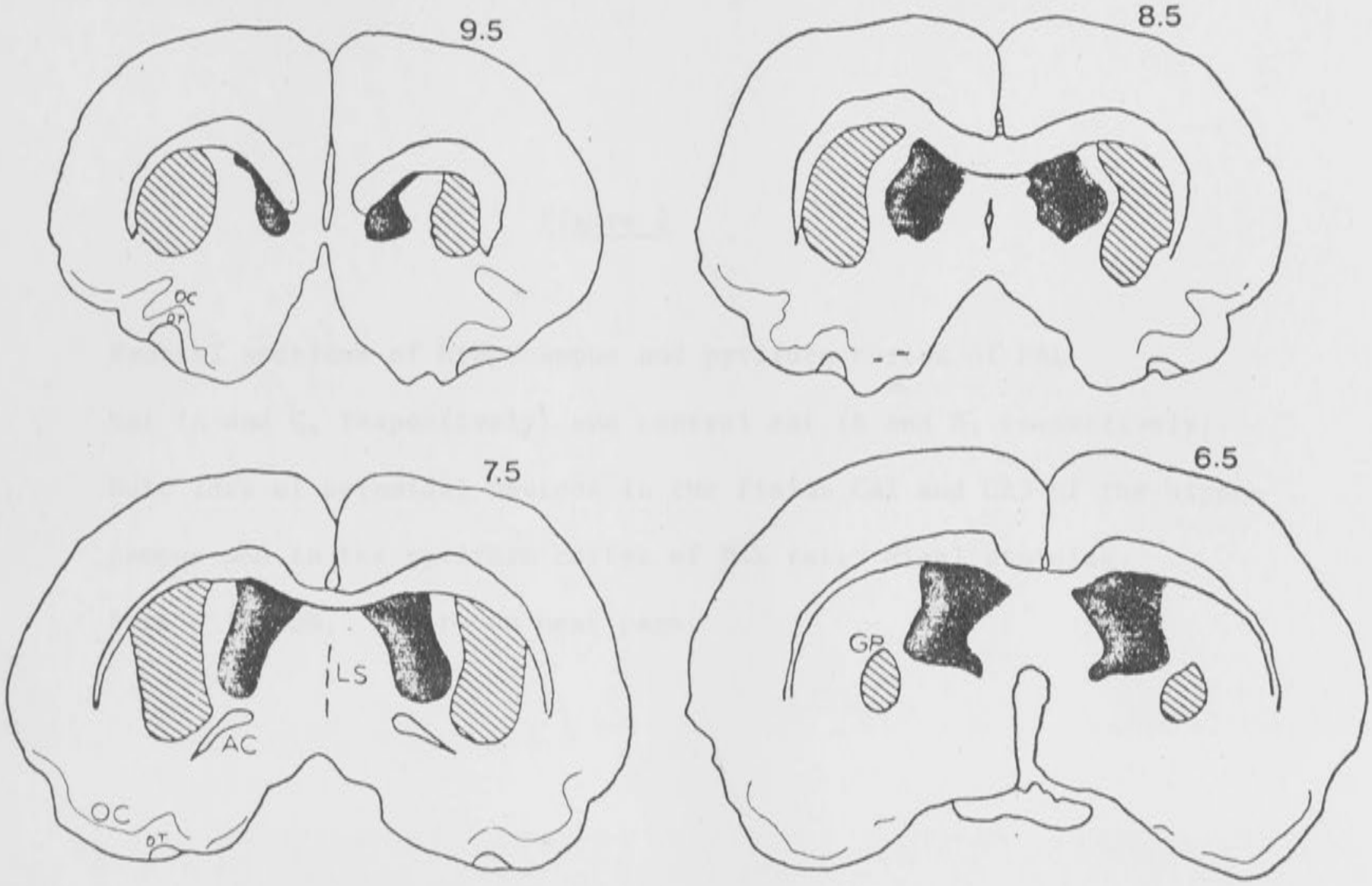

Figure 1. Reconstruction of volume of neuronal loss (hatched areas) in the striatum of representative rat with bilateral injections of three nmoles of kainic acid (KAL rat). Numbers correspond to frontal planes of the König and Klippel atlas (1963). AC, anterior commissure; GP, globus pallidus; LS, lateral septum; OC, olfactory cortex.

Figure 2

Frontal sections of hippocampus and pyriform cortex of KAL rat (A and C, respectively) and control rat (B and D, respectively). Note loss of pyramidal neurons in the fields CA1 and CA3 of the hippocampus and in the pyriform cortex of KAL rat. Nissl staining. Bars = 180 µm. Figure on next page.

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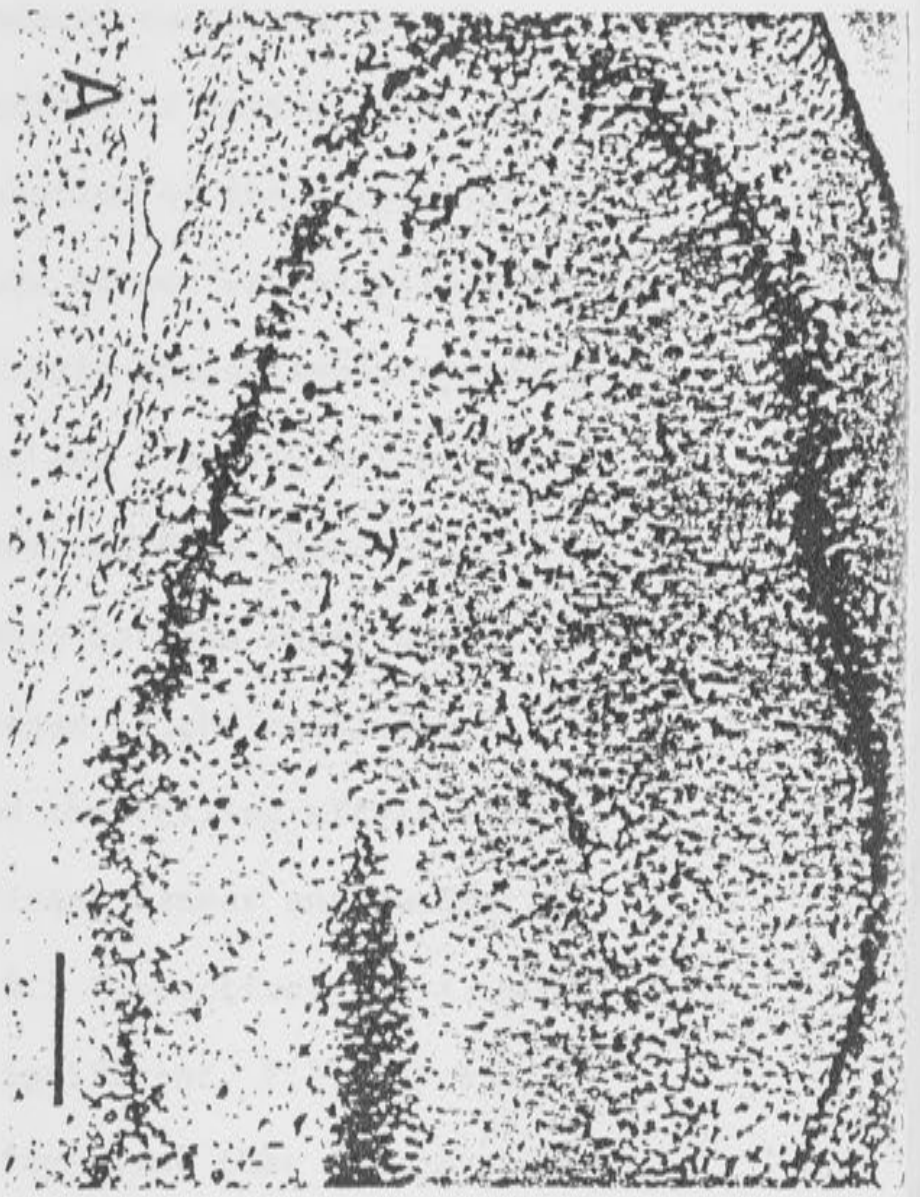
C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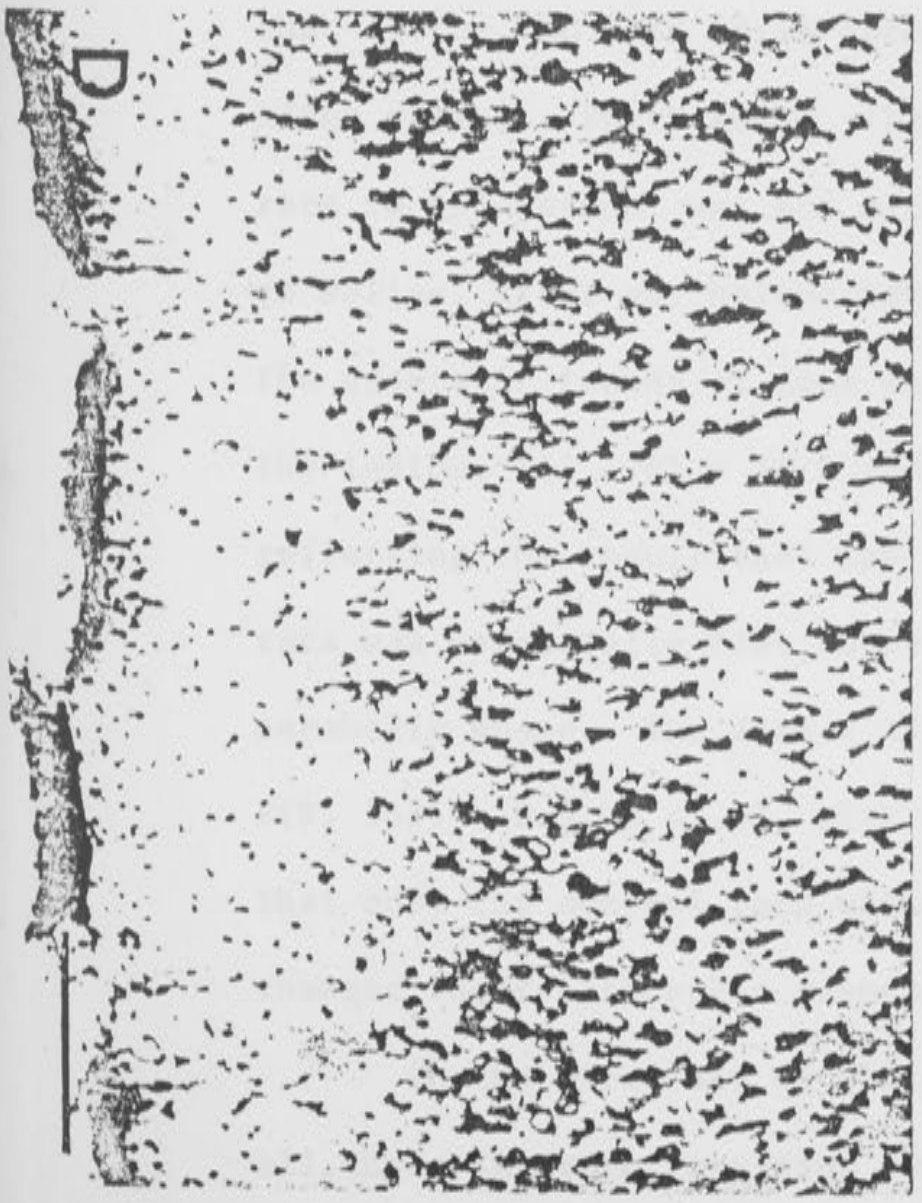
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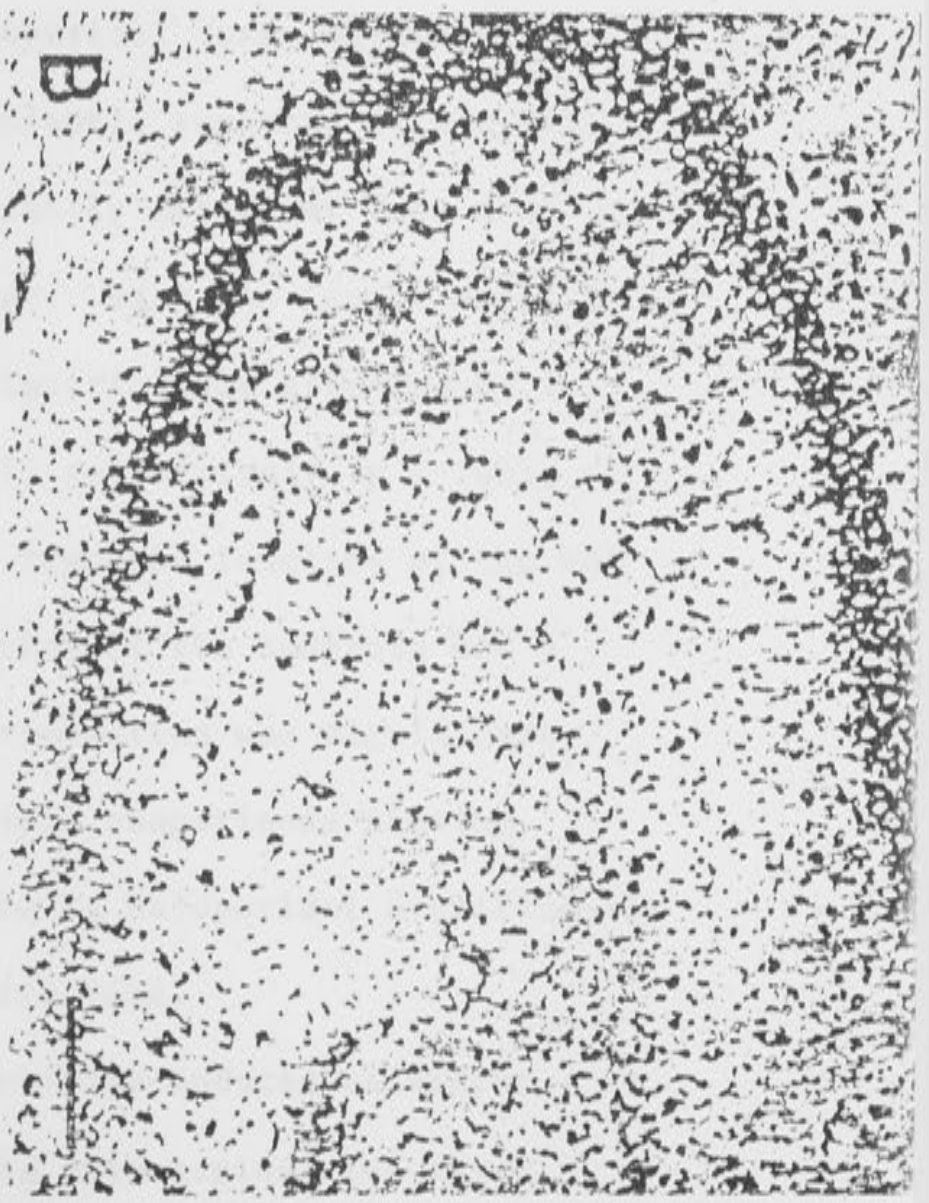
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3.2 *Catalepsy*

The results of haloperidol-induced catalepsy are shown in Figure 3. The significant main effect of lesion, $F(1, 18) = 14.0$, $p < .001$, reflected the marked attenuation of the cataleptic effect in the KAL rats. The dose main effect was significant, $F(3, 54) = 23.3$, $p < .0001$, with multiple comparisons revealing that the cataleptic effects of saline and 0.5mg/kg haloperidol significantly differed from each other and from those of 1 and 2mg/kg haloperidol. The difference between these two doses was not significant. The increase of the cataleptic effect with doses up to 1.0mg/kg, and the ceiling effect with 2mg/kg, were reflected by the significant linear and quadratic trends of dose $F(1, 18) = 65.8$ and 18.5 respectively, $p < .0001$. The lesion x dose interaction was significant, $F(3, 53) = 8.2$, $p < .001$, with multiple comparisons revealing that the cataleptic response of the control rats was significantly greater than that of the KAL rats after each haloperidol dose, although not after saline. Furthermore, the cataleptic response of the KAL rats to haloperidol was not significantly different from that to saline, after any haloperidol dose. Both the linear and the quadratic trends of dose were significantly affected by the lesion, $F(1, 18) = 18.0$ and 12.2, respectively, $p < .005$, reflecting the result that the dose response function of the KAL rats was essentially flat, whereas that of the controls had a parabolic shape. The dose x time interaction was significant, $F(9, 162) = 2.5$, $p < .01$, with multiple comparisons showing that only the control rats with 2.0mg/kg haloperidol significantly changed their cataleptic response with time.

On the first day of the pilocarpine-induced catalepsy all three KAL rats receiving 100mg/kg pilocarpine, and three

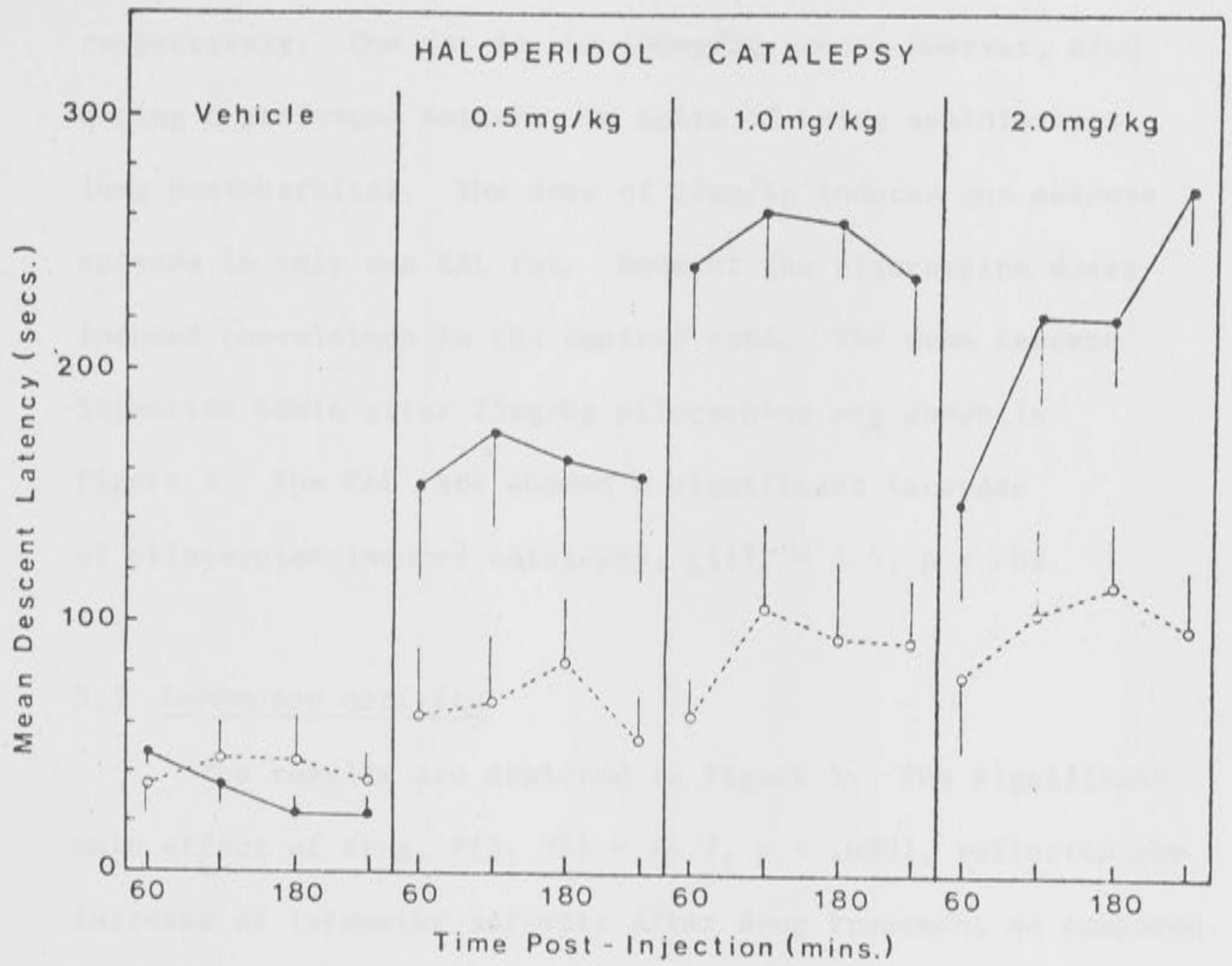


Figure 3. Catalepsy, measured as descent latency in the rod test, after saline (vehicle), 0.5, 1.0, and 2.0 mg/kg haloperidol, in 10 KAL (dotted lines) and 10 control (solid lines) rats. Analysis of variance revealed significant group differences for all haloperidol doses. Error bars represent standard errors of the means.

of the four KAL rats receiving 50mg/kg pilocarpine, showed seizures within 15min of the injection. The seizures were clonic and each episode lasted for about 30sec. Rats in the 100 and 50mg/kg groups had about 3 and 1 convulsions respectively. One rat in the 100mg/kg group, however, died during a prolonged seizure, in spite of being administered 16mg pentobarbital. The dose of 25mg/kg induced one seizure episode in only one KAL rat. None of the pilocarpine doses induced convulsions in the control rats. The mean descent latencies 60min after 25mg/kg pilocarpine are shown in Figure 4. The KAL rats showed a significant increase of pilocarpine-induced catalepsy, $t(17) = 2.4$, $p < .03$.

3.3 *Locomotor activity*

The results are depicted in Figure 5. The significant main effect of drug, $F(2, 32) = 84.7$, $p < .0001$, reflected the increase of locomotor activity after drug treatment as compared with the activity after saline. Multiple comparisons showed that both scopolamine and d-amphetamine significantly increased locomotor activity compared with saline, and that d-amphetamine did so significantly more than scopolamine.

The significant main effect of lesion, $F(1, 16) = 22.4$, $p < .005$, reflected the higher locomotor activity of the KAL rats compared with the controls. However, the lesion x drug interaction was also significant, $F(2, 32) = 8.7$, $p < .001$, with multiple comparisons revealing that i) the KAL rats locomoted significantly more than the controls after both scopolamine and d-amphetamine, although not after saline; ii) the KAL rats locomoted significantly more after scopolamine than after saline, whereas the controls did not;

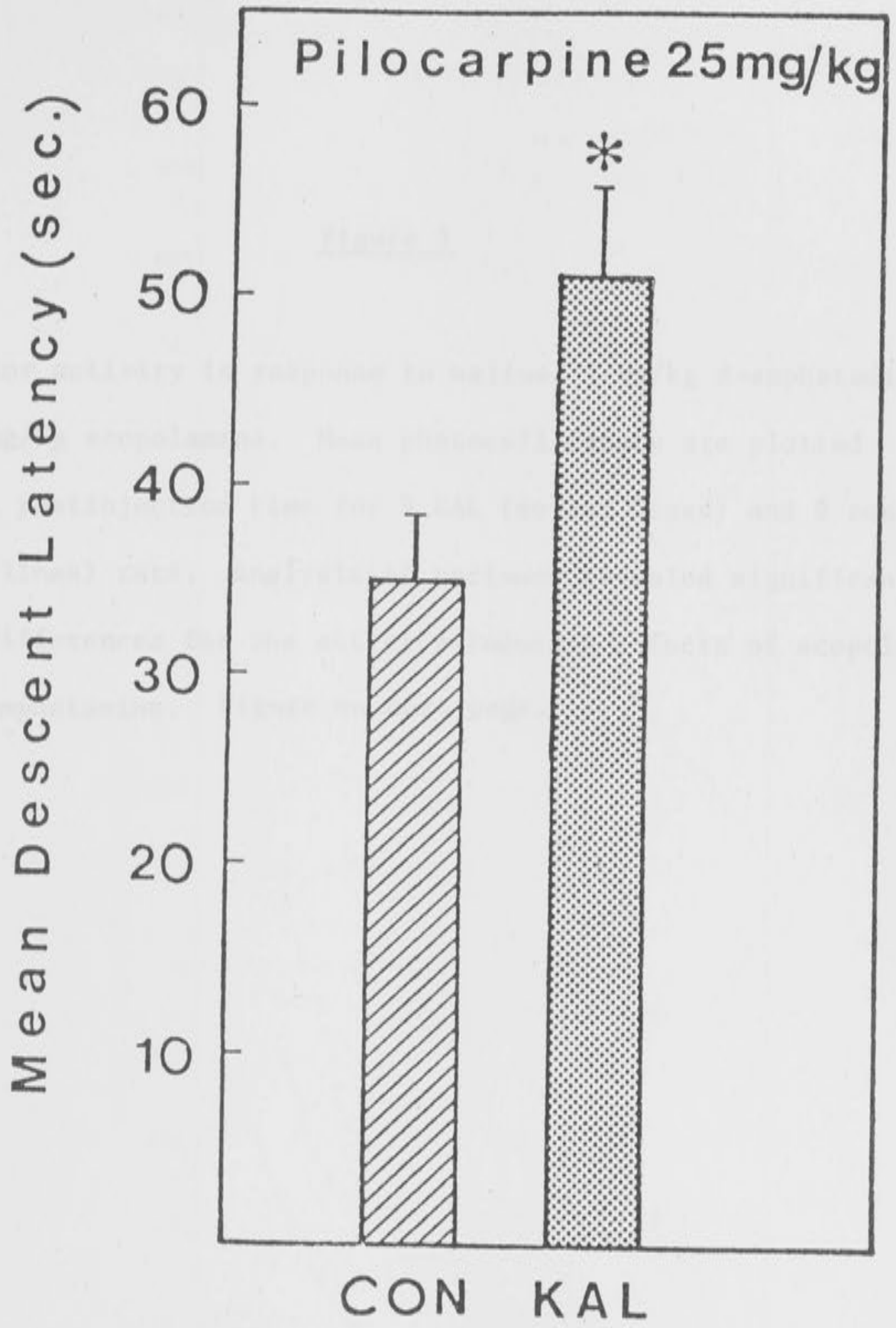
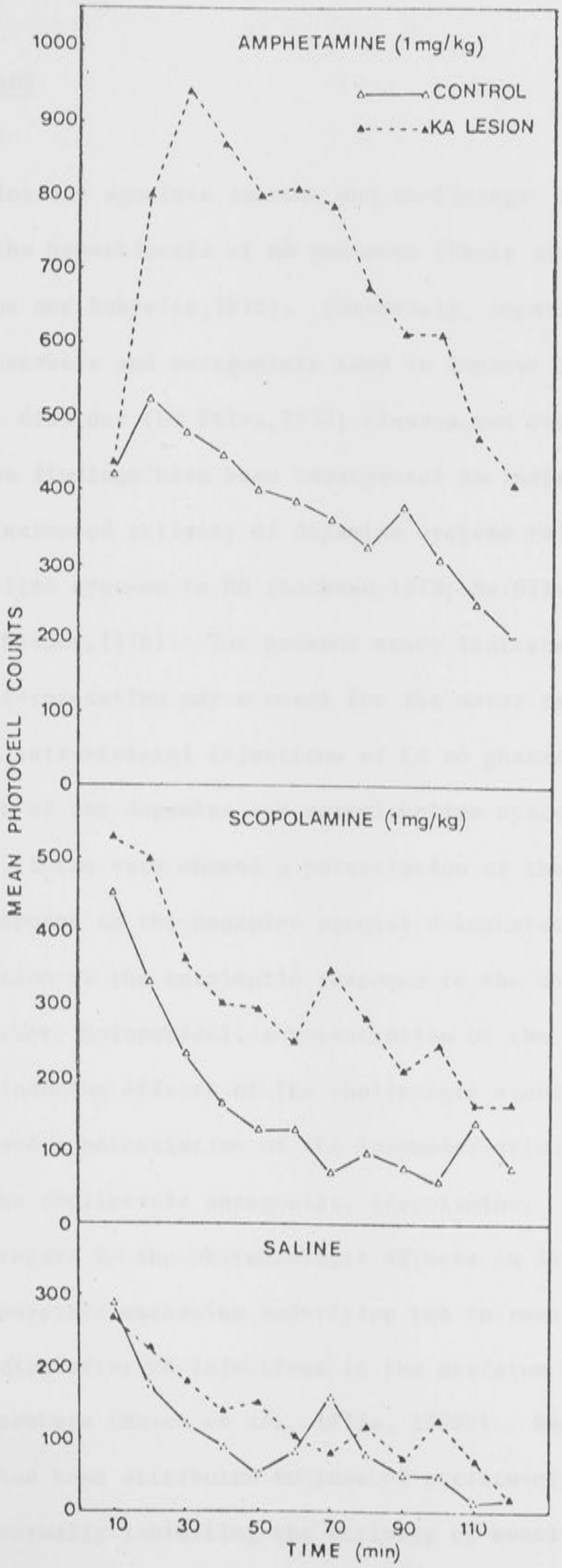


Figure 4. Mean catalepsy scores of 9 KAL and 10 control rats after parenteral administration of 25 mg/kg pilocarpine. Error bars represent standard errors of the means. *significantly differenct from controls, $p < .05$.

Figure 5

Locomotor activity in response to saline, 1 mg/kg d-amphetamine and 1 mg/kg scopolamine. Mean photocell counts are plotted against postinjection time for 9 KAL (dotted lines) and 9 control (solid lines) rats. Analysis of variance revealed significant group differences for the activity-inducing effects of scopolamine and d-amphetamine. Figure on next page.


AMPHETAMINE (1mg/kg)
CONTROL
KA LESION
SCOPOLAMINE (1mg/kg)
SALINE
MEAN PHOTOCELL COUNTS
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900
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500
400
300
200
100
0
500
400
300
200
100
0
300
200
100
0
10
30
50
70
90
110
TIME (min)

iii) both groups showed significantly higher activity after d-amphetamine than after saline.

4. DISCUSSION

Cholinergic agonists improve and cholinergic antagonists exacerbate the hyperkinesia of HD patients (Davis and Berger, 1978; Klawans and Rubovits,1972). Conversely, dopamine agonists exacerbate and antagonists tend to improve the hyperkinetic disorder (De Silva,1977; Klawans and Weiner,1974, 1976). These findings have been interpreted to indicate an abnormally increased activity of dopamine systems relative to acetylcholine systems in HD (Barbeau,1973; De Silva,1977; Klawans and Weiner,1976). The present study indicates that a similar interpretation may account for the motor response of rats with intrastriatal injections of KA to pharmacological manipulations of the dopamine and acetylcholine systems. Specifically, these rats showed a potentiation of the locomotor response to the dopamine agonist d-amphetamine, a marked reduction of the cataleptic response to the dopamine receptor blocker, haloperidol, a potentiation of the cataleptic and seizure-inducing effects of the cholinergic agonist pilocarpine and a potentiation of the locomotor-stimulating effects of the cholinergic antagonist, scopolamine.

With regard to the pharmacologic effects on locomotor activity, a possible mechanism underlying the increased response to d-amphetamine after KA injections in the striatum has been discussed elsewhere (Mason *et al.*, 1978a, 1978b). Briefly, this effect has been attributed to loss of striato-nigral projections normally inhibiting the activity of mesolimbic

dopaminergic neurons, which have been shown to be involved in facilitation of rat locomotor activity (Pijnenburg *et al.*, 1976). The presumed hyper-responsive mesolimbic dopamine system may also be involved in the enhanced locomotor response to scopolamine of KAL rats. Thus, Watanabe *et al.* (1978) demonstrated that scopolamine-induced locomotor activity is dependent upon intact dopamine systems. Costall *et al.* (1979) showed an antagonistic relationship between acetylcholine and dopamine within the nucleus accumbens with respect to locomotor activity. Stephens and Herberg (1979) found that scopolamine injected in the nucleus accumbens was especially effective in blocking neuroleptic antagonism of hypothalamic self-stimulation, and Snyder (1976) has suggested that anticholinergic agents might counteract the action of neuroleptic drugs by inhibiting the reuptake of dopamine, thus increasing its availability at receptor sites.

Although there is increasing evidence that locomotor activity is influenced by the mesolimbic dopamine system (Costall *et al.*, 1979; Pinjenburg *et al.*, 1976), neuroleptic-induced catalepsy appears to be more dependent on the nigro-striatal dopamine system. This assumption is primarily based on the finding that electrolytic lesions of the striatum, but not electrolytic lesions of the nucleus accumbens, greatly reduce neuroleptic-induced catalepsy (Costall and Olley, 1978; Fogg *et al.*, 1970; Honma and Fukushima,1978; Koffer *et al.*, 1978). Within the striatum, neuroleptics such as haloperidol act at dopamine receptor sites (Creese *et al.*, 1975, 1976). Intra-striatal injections of KA destroy dopamine receptors (Gorani *et al.*, 1978; Schwarcz *et al.*, 1978). Thus, the attenuation of haloperidol-induced catalepsy in KAL rats is probably due

to loss of these dopamine receptors. On the other hand, since the dopamine receptors of the nucleus accumbens are presumably still intact, it might be expected that KAL rats would still exhibit neuroleptic antagonism of locomotor activity mediated by the mesolimbic dopamine system. In fact, Mason and Fibiger (1979) demonstrated that the neuroleptic pimozide was equally effective in reducing nocturnal locomotor activity of KAL and control rats. In HD patients, neuroleptics and cholinergic agonists exert effects in comparable directions, however, in the present study with KAL rats, the cholinergic agent pilocarpine produced effects opposite to those elicited by the dopaminergic antagonist haloperidol. The differential effects of neuroleptics on nocturnal locomotor activity (Mason and Fibiger, 1979) and catalepsy (the present study) in KAL rats suggests that the effects of such drugs on hyperkinesia in HD patients may be similar to the former behavioural findings in KAL rats. This intimates that HD hyperkinesia may be underlied by enhanced dopaminergic neurotransmission in the mesolimbic projection to the nucleus accumbens, possibly as a result of disruption of feedback inhibition from the striatum (Mason *et al.*, 1978a, b). Indeed, Spokes (1980) recently demonstrated highly significant increases in dopamine concentrations in the nucleus accumbens of post-morten HD brain tissue.

The potentiation of pilocarpine-induced catalepsy in KAL rats is similar to that obtained by Costall and Olley (1971) after administration of arecoline in rats with electrolytic lesions of the striatum. Also, Silbergeld and Hruska (1979) showed that rats with KA-induced striatal neurodegeneration are especially sensitive to the tremorogenic, rotational and seizure-inducing effects of arecoline and tremorine. KA injections in the striatum reduce the number

of striatal muscarinic receptors without changing the affinity of the remaining binding sites (Hruska *et al.*, 1978). Silbergeld and Hruska (1979) thus suggested that intrastriatal KA injections may increase the sensitivity of cholinergic receptors in non-striatal areas as a result of nonspecific damage. This interpretation is possible, in view of the damage to the hippocampus, pyriform cortex and neocortex found in most of the KAL rats of the present study. However, it should be noted that a KAL rat with little or no extrastriatal damage showed two seizures after 100mg/kg pilocarpine. Furthermore, this rat was not appreciably different from the other KAL rats in the other behavioural tests. Nonetheless, the multiple sites of KA-induced brain lesions impose a degree of caution in the anatomical interpretation of the behavioural results.

Pisa *et al.* (1980a, b) have suggested that the multifocal neurodegenerative effects of intrastriatal KA injections may enhance the analogy of this animal preparation with HD, because they closely resemble the diffuse neuropathology of HD, at least in its advanced stages (Bruyn, 1968; Forno and Jose, 1973; Roizin *et al.*, 1976). In fact, Coyle *et al.* (1977) suggested additional injections of KA in the globus pallidus and the cortex be given to obtain a more faithful model of the pathologic sequelae of HD, after they failed to detect extrastriatal damage in their KAL rats. The present results add to the growing number of recent pharmacological studies (Borison and Diamond, 1979; Mason *et al.*, 1978a, b; Mason and Fibiger, 1979; Owen 1980; Silbergeld and Hruska, 1979) which support the view that this animal model can be valuable for possible pharmacotherapies in HD.

Acknowledgements

We appreciate the excellent technical assistance of Ms. Stella Atmadja for histology, Ms. Barbara Piper for typing and Dr. Julia Irwin for statistical analysis. The helpful comments of Drs. I.G. Morgan, I. Creese, J. Irwin and R.F. Mark on the manuscript are also greatly appreciated. This work was supported, in part, by the Medical Research Council of Canada. P.R. Sanberg is an ANU Research Scholar and M. Pisa is a MRC Fellow.

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Reprinted from the Archives of Neurology
June 1979, Volume 36

Sedative Effects of Apomorphine in an Animal Model of Huntington's Disease

Paul R. Sanberg, MSc; John Lehmann; Hans C. Fibiger, PhD

• **The sedative effectiveness of apomorphine in a newly developed animal model of Huntington's disease was examined. The motor responses of rats with kainic acid lesions of the neostriatum to a sedative dose of apomorphine (50 μg/kg) was similar to that observed in intact controls. In contrast, compared to controls, a marked potentiation of the motor stimulant effects of dextroamphetamine was confirmed in the kainic acid-lesioned group. We suggest that the pathological changes underlying the symptoms observed in this animal model and in Huntington's disease do not include abnormalities in presynaptic dopamine receptors in the neostriatum.**

(*Arch Neurol* 36:349-350, 1979)

Recently, Corsini and colleagues[1] demonstrated that low doses of apomorphine hydrochloride produced a marked improvement in the abnormal involuntary movements of patients suffering from Huntington's disease (HD). These results further support those of previous investigators[2,3] who showed that apomorphine does not exacerbate the involuntary movements, as might be predicted since apomorphine is a dopamine agonist, but instead exhibits a paradoxical beneficial effect in such patients. As pointed out by Corsini et al[1] and Tolosa,[3] this effect of low doses is presumably mediated by stimulation of presynaptic dopamine (DA) receptors, ie, DA autoreceptors. It has also been demonstrated by Corsini et al[1] and Carrol et al[2] that low doses of apomorphine also produce sedative effects in normal, schizophrenic, and tardive dyskinetic subjects. However, it is not known how this response of patients with HD to sedative doses of apomorphine compares with a control population of subjects.

Recently, we have shown that an animal model (produced by lesioning the neostriatum with kainic acid) that is biochemically,[4] histologically,[7] and behaviorally[8] similar to HD also responds to pharmacological agents in a manner very analogous to that of patients with HD.[9,10] When the action of stereotypy-producing doses of amphetamine and apomorphine were compared on these animals, it was demonstrated that while amphetamine-induced stereotypy was enhanced in kainic acid-lesioned (KAL) rats compared to control rats, apomorphine-induced stereotypy did not differ significantly between groups. Maj et al[11] recently demonstrated that, as in humans, low doses of apomorphine have a sedative action on rats, the maximum effect occurring with 50 μg/kg. We were interested in determining how the KAL animals that mimic patients with HD respond to a sedative dose of apomorphine. By comparing the sedative effects of apomorphine between this animal model of HD and control rats, it may be possible to examine changes in the state of the DA autoreceptors (ie, supersensitive or subsensitive) in this animal model, and by inference in HD.

METHODS

In nine male Wistar rats (weighing about 300 g each), 3 nmoles of kainic acid in 0.5 μl of phosphate-buffered saline (pH, 7.4) were stereotaxically injected into each neostriatum as described elsewhere.[4-10] Nine control rats received injections of the vehicle only. Six weeks later, all animals were injected intraperitoneally with either 50 μg/kg of apomorphine hydrochloride or the same volume of saline in a randomly paired fashion. One week later, the other drug solution was administered. The experiment was run blind insofar as the drug solutions were coded so that the investigator did not know their identity. Ten minutes following the drug administration, each animal was placed separately into photocell activity cages and his locomotor activity was recorded for 20 minutes. The photocell cages measured 61 cm in diameter, were painted black internally, and each was transected by six infrared photocell beams, interruption of which incremented electromechanical counters at a distance from the cages. Photocell beam interruptions were cumulated over five-minute periods and then recorded by an automatic printout counter.

RESULTS

As Fig 1 shows, KAL and control animals demonstrated marked depression of mean locomotor activity compared to their control activities (saline) following injection of 50 μg/kg of apomorphine hydrochloride ($P < .01$). There were no significant differences, however, between the groups in this depressant effect of apomorphine ($P > .10$). Two weeks later, all animals were habituated to the photocell cages for one hour and then given 1 mg/kg of dextroamphetamine sulfate (intraperitoneally). Their amphetamine-induced locomo-

Accepted for publication May 27, 1978.

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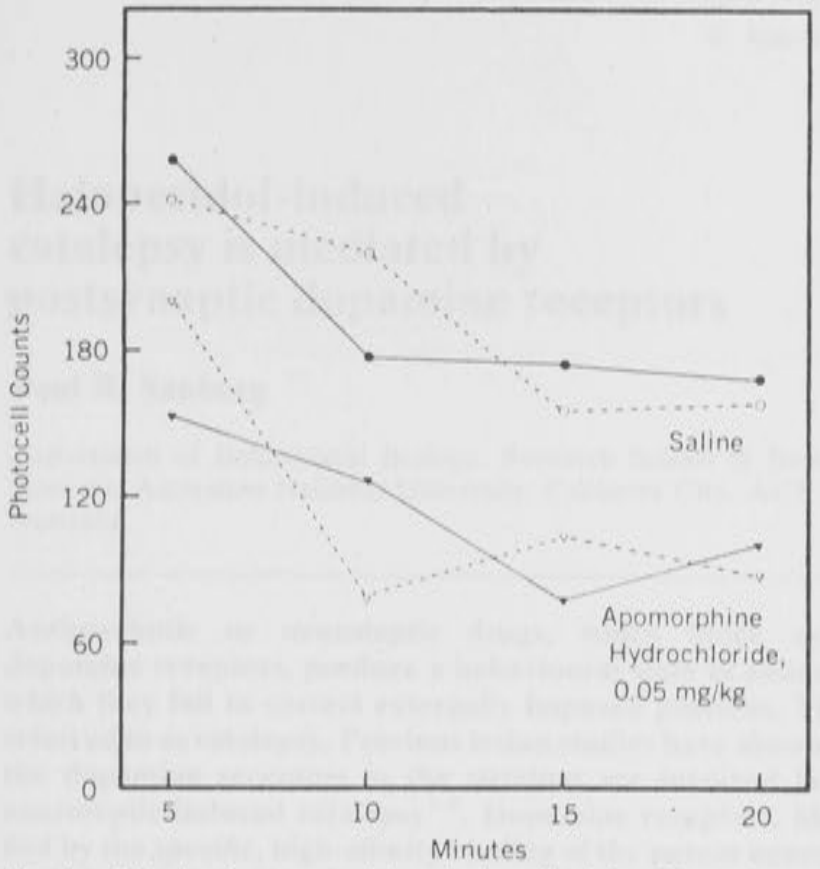

Fig 1.—Effect of apomorphine hydrochloride, 50 μg/kg (triangles), and saline (circles) on locomotor activity in kainic acid-lesioned (KAL) rats (broken lines) and control rats (solid lines). Analyses of variance disclosed significant difference ($P < .01$) between treatments (apomorphine vs saline) and no significant differences ($P > .10$) between groups (KAL vs control). Data represent means of nine animals in each group.

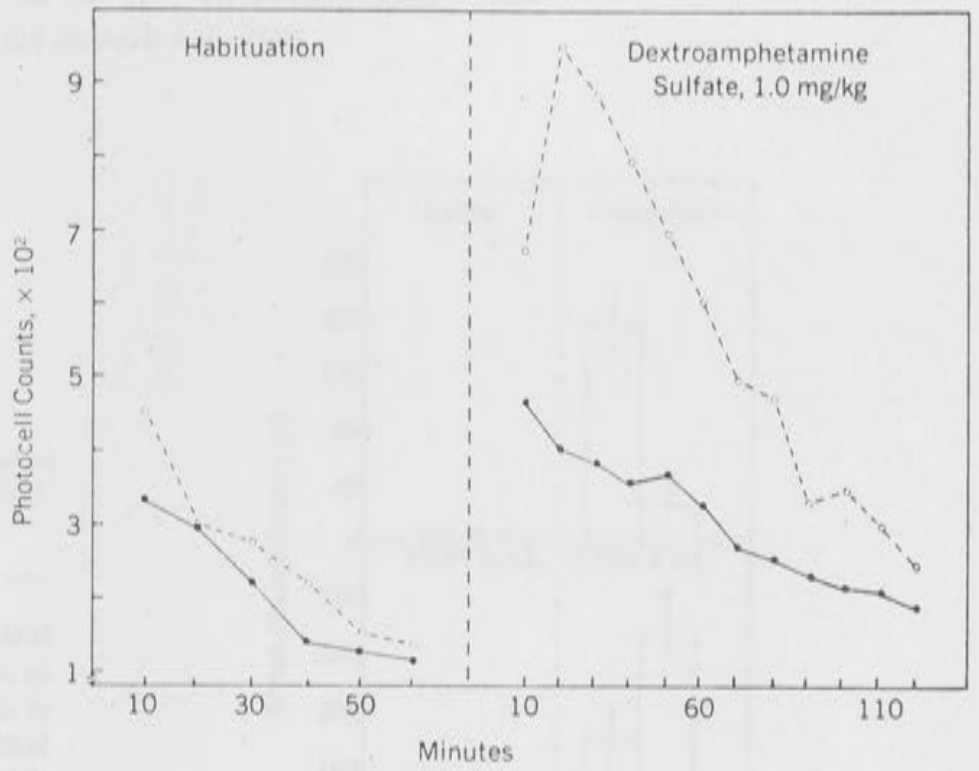

Fig 2.—Effect of dextroamphetamine sulfate (1 mg/kg) on mean locomotor activity in kainic acid-lesioned (KAL) rats (broken lines) and control rats (solid lines). Analysis of variance disclosed no significant differences between groups (KAL vs control) during habituation ($P > .10$). Following dextroamphetamine administration, however, KAL animals showed significantly greater increase in mean locomotor activity ($P < .001$) than did controls.

tor response was assessed for two hours as described previously. As Fig 2 shows, there were no significant differences ($P > .10$) in mean spontaneous locomotor activity in the photocell cages over one hour. However, compared to controls, a very marked enhancement ($P < .001$) in amphetamine-induced locomotor activity was observed in the KAL animals. This potentiated response following amphetamine administration confirms previous findings[9] and its importance in terms of the animal model of Huntington's disease has been elucidated elsewhere.[9] Histological analysis showed that most of the neuronal cell destruction was confined to the neostriatum. Shrinkage of the neostriatum, ventricular dilation, and neostriatal glial proliferation were also evident.

COMMENT

These results demonstrate that in rats that mimic neuropathologically and behaviorally those conditions occurring in Huntington's disease, their response to a sedative dose of apomorphine is of the same magnitude as is found in normal animals. These results suggest, therefore, that neostriatal DA autoreceptors in both the KAL animal model and in HD are normal and that the pathological changes underlying the symptoms observed in HD do not include abnormalities in presynaptic DA receptors in the neostriatum. The present data further suggest that additional studies are warranted concerning the effects of DA agonists in the treatment of Huntington's disease.

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Printed and Published in the United States of America

Reprinted from Nature, Vol. 284, No. 5755, pp. 472-473, April 3 1980

Haloperidol-induced catalepsy is mediated by postsynaptic dopamine receptors

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Antipsychotic or neuroleptic drugs, which block central dopamine receptors, produce a behavioural state in animals in which they fail to correct externally imposed postures. This is referred to as catalepsy. Previous lesion studies have shown that the dopamine receptors in the striatum are involved in this neuroleptic-induced catalepsy[1-5]. Dopamine receptors, identified by the specific, high-affinity binding of the potent neuroleptic haloperiodol, have been shown to be equally distributed postsynaptically on striatal neurones and presynaptically on cortico-striatal terminals[6-9]. Because the electrolytic lesioning studies[1-5] have unavoidably damaged both pre- and postsynaptic striatal dopamine receptors, it is not known whether these two receptors are separately involved in neuroleptic-induced catalepsy. Using kainic acid and cortical ablation to destroy postsynaptic and presynaptic dopamine receptors, respectively[6-9], the present study demonstrates that the cataleptic effects of haloperidol are apparently mediated by dopamine receptors localised postsynaptically on striatal neurones.

Ten male Wistar hooded rats weighing about 350 g received 6 nmol of kainic acid injected stereotaxically into each striatum[10,11]. Ten control animals received vehicle injections only. In another eight rats the parietal and frontal cortex was removed. Nine control animals received sham operations. All the rats received a prophylactic subcutaneous injection of aquacaine (0.2 ml) and an intraperitoneal (i.p.) injection of diazepam (5 mg per kg) immediately following surgery (diazepam reduces the possibility of extrastriatal damage in rats injected with kainic acid[12,13]). All animals were allowed to recover for 4 weeks before testing for catalepsy. After testing, the animals were perfused intracardially with 10% formol saline and their brains examined histologically. Consistent with previous reports[10,11,14,15], the kainate-lesioned rats showed a considerable loss of neuronal cell bodies in the dorsal two-thirds of the striatum, whereas very little extrastriatal damage was apparent. In all rats with cortical ablation there was no destruction below the level of the corpus callosum.

The cataleptic effect of the central dopamine receptor blocker haloperidol (1 mg per kg) on kainate-lesioned and cortex-ablated rats is shown in Fig. 1. Catalepsy was measured by gently placing the animal's forepaws on a horizontal bar located 8.0 cm above the floor and timing the period before the animal placed at least one paw on the floor. If an animal remained on the bar for more than 5 min, it was removed and a score of 300 s was given. After saline injections, rats in all groups typically removed their paws from the bar within 20 s. The decorticate rats, however, showed slightly higher descent latencies under saline compared with their control group ($P < 0.05$). This may be due to an increased emotionality, as they defaecated substantially more than controls during testing. Injection of haloperidol induced considerable catalepsy in the control animals (Fig. 1). Haloperidol-induced catalepsy was almost totally blocked in the kainate-lesioned rats ($P < 0.01$). By contrast, haloperidol tended to increase catalepsy in decorticate rats, although this increase did not reach significance ($P < 0.07$).

It has previously been demonstrated[6-9] that kainic acid-induced striatal lesions destroy about 40% of ^{3}H-haloperidol binding sites in the striatum, demonstrating postsynaptic localisation on intrinsic striatal neurones. Decortication, which removes the cortico-striatal tract, resulted in a similar reduction of ^{3}H-haloperidol binding, showing presynaptic localisation of dopamine receptors on cortico-striatal afferents. Although previous studies[1-5] have shown that haloperidol-induced catalepsy seems to be mediated by dopamine receptors in the striatum, the electrolytic lesioning techniques used have been unable to examine specifically the roles of these two dopamine receptors in mediating the behavioural response. My results show that the cataleptic response to haloperidol injection is almost completely abolished in kainic acid-lesioned rats. The marginal increase in catalepsy induced by haloperidol in these animals may be due to the fact that destruction of the striatal interneurones is incomplete. If anything, haloperidol is more effective in inducing catalepsy in decorticate animals. This suggests that haloperidol-induced catalepsy requires the binding of haloperidol to postsynaptic dopamine receptors in the striatum[6-9]. The present findings may have relevance for understanding differences in antipsychotic potency of neuroleptic drugs in diseases with cortical and/or striatal atrophy.

I thank Professors R. F. Mark and I. Creese and Drs I. G. Morgan, D. Ehrlich, M. Pisa and I. J. Faulks for helpful comments, Roche (Australia) for the gift of diazepam, and Janssen (Belgium) for the gift of haloperidol. The author is an ANU Research Scholar.

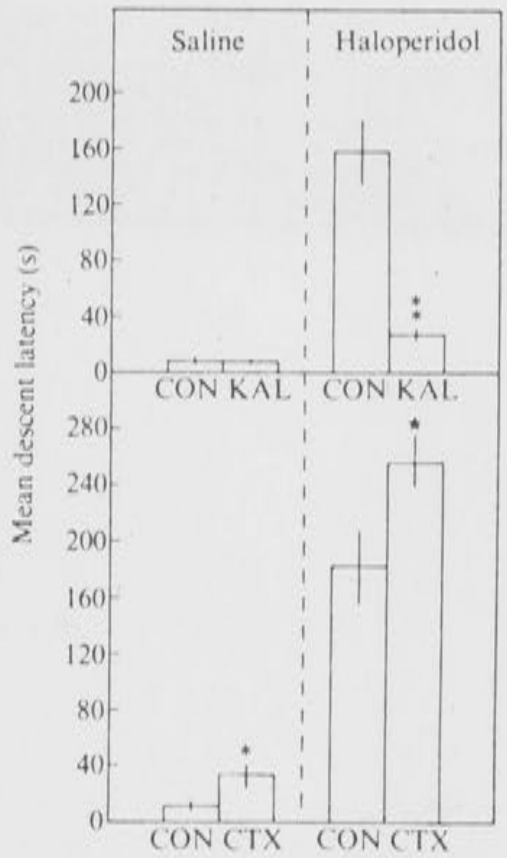

Fig. 1 Catalepsy induced in control (CON), kainate-lesioned striatal (KAL) and cortically ablated (CTX) rats. KAL rats ($n = 10$) were injected with 6 nmol kainate dissolved in 1.0 μl of phosphate-buffered isotonic saline solution, *p*H 7.2, as described elsewhere[10,11]. CON rats ($n = 10$) received infusions of the vehicle. CTX rats ($n = 8$) were anaesthetised with sodium pentobarbital (50 mg per kg i.p.) and positioned on a Kopf stereotaxic instrument. The skin was reflected, the calvaria overlying the parietal and frontal cortex was removed, and the underlying cortex was removed to the level of the corpus callosum by aspiration; bleeding was controlled by gelfoam. CON rats ($n = 9$) received sham operations. The data represent the pooled mean results of four catalepsy tests on each rat carried out at 1-h intervals following drug administration. Haloperidol was dissolved in 0.9% saline and injected i.p. at 1 mg per kg. All animals were injected with either haloperidol or saline in a randomly paired fashion. One week later, the other drug solution was administered. The experiment was run blind insofar as the drug solutions were coded. Control and lesioned groups differed at the following levels of significance using a two-tailed Mann–Whitney *U* test[16]: ★, 7%; *, 5%; **, 1%.

Received 15 November 1979; accepted 5 February 1980.

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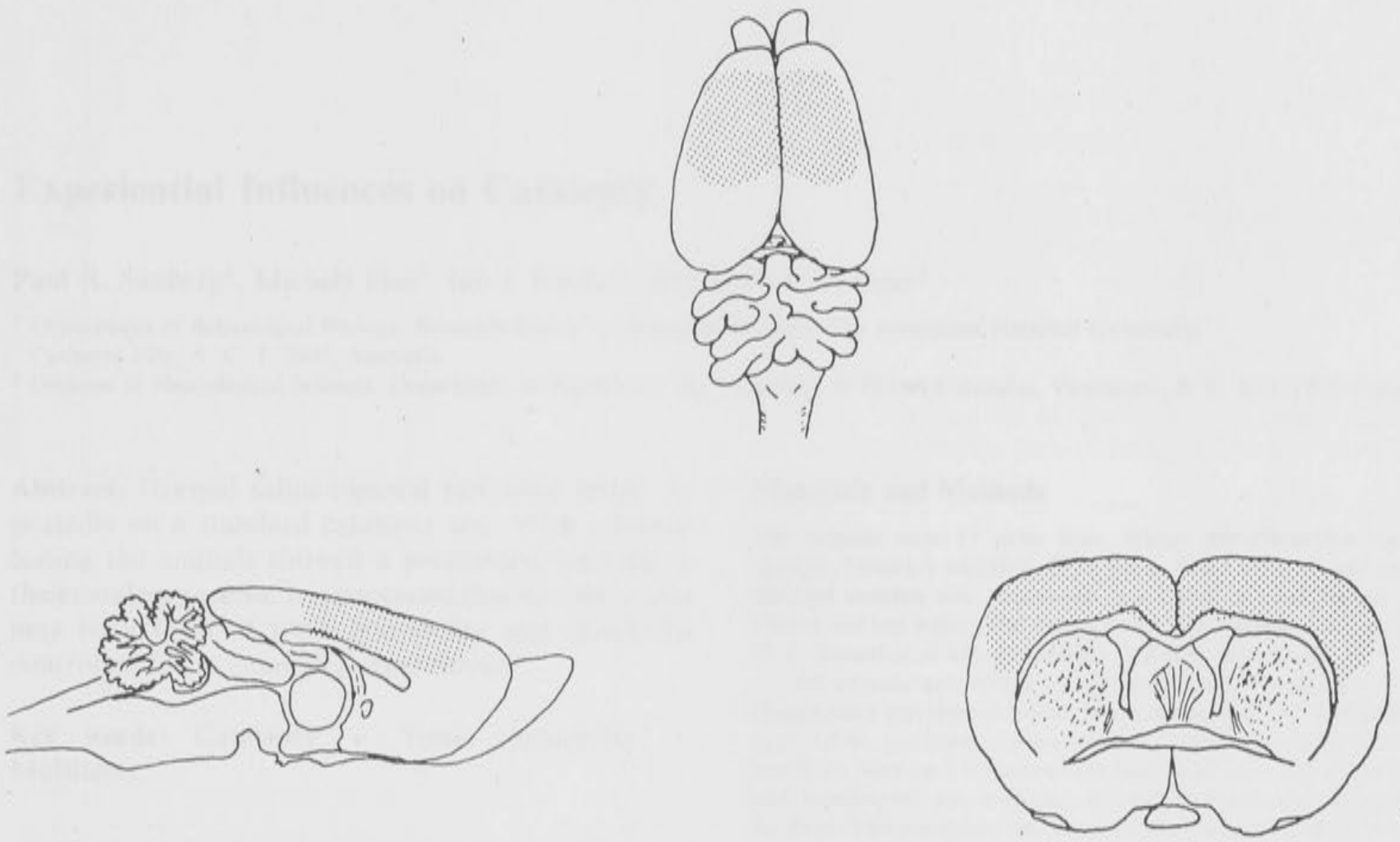

Figure 2. Pictorial illustration of representative cortical lesion produced by aspiration in rats. Number corresponds to frontal planes of König and Klippel atlas.

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Experiential Influences on Catalepsy

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Abstract. Normal saline-injected rats were tested repeatedly on a standard catalepsy test. With repeated testing the animals showed a progressive increase in their catalepsy scores. It is suggested that this behaviour may be a form of tonic immobility and should be controlled for in catalepsy experiments.

Key words: Catalepsy – Tonic immobility – Multitests

Catalepsy in laboratory animals is usually defined as inability to correct externally imposed postures. It is invariably measured in terms of the time elapsed before the posture is corrected. The simplicity of this technique makes it an excellent behavioural tool for investigating the functional state of neurochemical systems known to influence catalepsy. Recently, Stanley and Glick (1976) demonstrated that if repeated catalepsy tests were given to the same animals, haloperiodol-induced catalepsy was greater in later tests. However, Costall et al. (1978) could not replicate these findings, and therefore argued against the proposal of Stanley and Glick (1976) that experience can affect the duration of catalepsy.

In the process of evaluating the effects of striatal lesions on haloperiodol- and pilocarpine-induced catalepsy (Sanberg et al., 1979), we noticed that the saline-injected group had unusually high catalepsy scores on a standard catalepsy test. Further analysis suggested that this was a result of previous experience of the animals in the catalepsy test. In view of the discrepancy between previous investigations, and to verify our preliminary observation that catalepsy which is not drug-induced can increase with experience, the present study was performed.

Offprint requests to: P. R. Sanberg

Materials and Methods

The subjects were 11 naive male Wistar rats (Woodlyn Farms, Guelph, Ontario) weighing 275 – 325 g. They were housed in individual stainless steel cages with free access to food (Purina Rat Chow) and tap water. The colony room had a temperature of 22 – 25° C, humidity of 45 – 55% and a 12 h light-dark cycle.

All animals were tested for catalepsy using a standard bar test (Kuschinsky and Hornykiewicz, 1972; Mason et al., 1978; Moleman et al., 1978). Catalepsy was measured by gently placing the animals' two front paws on a horizontal bar positioned 8 cm above the floor and recording the time in seconds before the animal put both paws on the floor. This procedure was repeated four times on each test day at 1-h intervals after IP administration of saline. The animals were kept in their home cages between measurements. All test days were separated by 2 nontest days.

Results

The results on the first 4 test days of repeated catalepsy measurements following saline injection are depicted in Fig. 1. Analysis of variance revealed that the apparent increase in descent latency over the first 4 test days was significant [$F(3,30) = 9.93$, $P < 0.01$]. On 3 additional test days the rats did not differ significantly in their descent latencies from those recorded on day 4 (data not shown).

Discussion

The present results demonstrate increased catalepsy in normal non-drugged rats following repeated testing experience, and support the findings of Stanley and Glick (1976). The increased catalepsy is not due to the repeated saline injections, because similar results are obtained with uninjected animals (Sanberg and Faulks, unpublished data). While Costall et al. (1978) did not detect experiential factors involved in catalepsy measurements, they did mention experiential enhancement reported by other workers (unreferenced) on normal rats tested for catalepsy using 'vertical wire

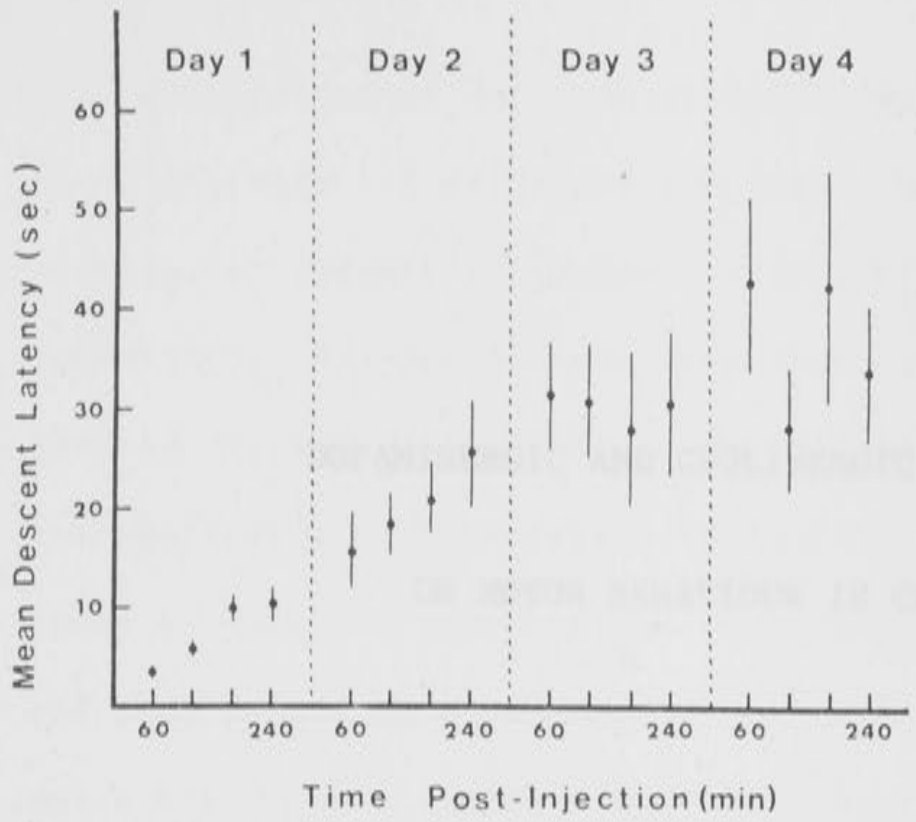

Fig. 1. Catalepsy following administration of saline over 4 testing days. Mean latency to place two front paws on floor is plotted against post-injection time for 11 rats. Error bars represent SEM

netting'. Our results confirm this statement, and extend it to include the bar test method of catalepsy, a technique more similar to that used by Costall et al. (1978).

Although Costall et al. (1978) have termed the experience-induced increase in catalepsy 'learning', it is possible that the phenomenon is a form of tonic immobility, which is known to be elicited in rats by only a few seconds of restraint (Bures et al., 1976). Thus the short handling prior to placing the animal on the bar during catalepsy measurement may be inducing tonic immobility. Tonic immobility in chickens may take several attempts to elicit (Gallup et al., 1976; Maser and Gallup, 1974) and seems to increase in duration during early repeated exposures (Faulks and Sanberg, unpublished observations). Tonic immobility is affected by a multitude of variables, such as animal strain (Gallup et al., 1976), the experimenter (Gallup et al., 1972), arousal (Kaufman and Rovee-Collier, 1978), age (Ratner and Thompson, 1960) and is used as an index of fear (Nash and Gallup, 1975). It is likely that the discrepancy between our results and those of Costall et al. (1978) is a function of differences in such variables.

As first reported by Stanley and Glick (1976), intensification of cataleptic behaviour by prior experience is a real phenomenon. It can occur in normal untreated animals. Therefore pharmacological investigations of catalepsy must include drug-injected and control animals matched for experience, as well as for all the more usual measures.

Acknowledgements. This work was supported by the Medical Research Council of Canada. We thank I. G. Morgan for comments on the manuscript. P. R. Sanberg and I. J. Faulks are A.N.U. Research Scholars. M. Pisa is a M.R.C. Fellow.

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Received January 24, 1980

DOPAMINERGIC AND CHOLINERGIC INFLUENCES ON MOTOR BEHAVIOUR IN CHICKENS.

Unpublished.

ABSTRACT

Stimulation of dopamine receptors by apomorphine enhanced stabilimeter motor activity and decreased the duration of tonic immobility in chickens. Blockage of dopamine receptors by haloperidol increased the duration of tonic immobility. Haloperidol was also shown to attenuate the apomorphine-induced increase in activity. Motor activity could also be increased by the cholinergic antagonist, scopolamine. In addition, scopolamine was shown to decrease the duration of tonic immobility. These results support the involvement of dopaminergic and cholinergic systems in controlling motor behaviour in birds. Furthermore, they suggest that tonic immobility and stabilimeter motor activity may be good behaviours for looking at the role of these neurochemical systems on motor behaviour in chickens.

Key words: Chickens, dopamine, acetylcholine, motor behaviour, apomorphine, haloperidol, scopolamine

Compared to mammals, the role of dopaminergic and cholinergic systems on motor behaviour in birds is little known. Stereotyped behaviours in birds, such as repetitive head-neck movements, compulsive preening, pecking and vocalization are induced by injections of the dopamine agonist, apomorphine (Nistico' and Stephenson, 1979) and seem to be mediated by the dopaminergic tegmentopaleostriatal system, the homologue of the dopaminergic nigrostriatal system in mammals (Goodman and Azzaro, 1978; Nistico' and Stephenson, 1979). While most studies have been performed with pigeons (Nistico' and Stephenson, 1979), chickens also show similar stereotyped behaviours following apomorphine injections (de Lanerolle and Youngren, 1978; Machlis, 1980; Osuide and Adejoh, 1973). As in mammals, chickens also show increased locomotor activity in response to the dopaminergic agonists, d-amphetamine (Spooner and Winters, 1966; Wallach *et al.*, 1972) and l-dopa (Wallach *et al.*, 1972). In all these experiments with chickens the increase in motor behaviour was recorded by methods of direct observation. Thus time course studies have been limited. Recently, Wallnau *et al.* (1979) measured activity increases on automatic stabilimeters after one dose of apomorphine for 10min. On the other hand, Wallnau *et al.* (1979) demonstrated that the dopamine receptor blocker, haloperidol, prolonged tonic immobility in chickens. This behaviour measure is thought to be similar to catalepsy commonly measured in rodents (Sanberg *et al.*, 1980).

The effects of cholinergic agents on motor behaviour in birds are less known than the effects of dopaminergic drugs. In rodents, anticholinergics such as scopolamine increase locomotor activity (Sanberg *et al.*, 1979). Two available studies which have been performed with chickens have shown enhanced activity, as measured in photocell activity cages, following scopolamine administration (Thompson *et al.*, 1974; Ksir, 1978). The reported effects of scopolamine on tonic immobility, however, have been contradictory. Thompson *et al.* (1974) and Woodruff *et al.* (1976) have

shown decreased tonic immobility following injections of scopolamine in chickens and ducks, respectively. Ksir (1978) on the other hand, found a lack of effect of scopolamine on tonic immobility in chickens.

In the present study, apomorphine, haloperidol and scopolamine were tested on stabilimeter activity and tonic immobility in order to clarify further the role of dopaminergic and cholinergic systems in motor behaviour in chickens.

METHODS

Animals and Housing

White Leghorn x Black Australorp male chickens were obtained from Research Poultry Farm (Victoria) on day 1 of life. They were housed in groups until one week before testing, at which time they were housed individually in 23 x 23 x 29cm metal cages with a perspex front. Chicks had free access to grain (Hutmill, Victoria) and water (tap water with terramycin added) during the study.

Drugs

Haloperidol (McNeill), apomorphine hydrochloride (Sigma) and scopolamine hydrobromide (Sigma) were dissolved in distilled water and injected in a volume of 1ml/kg. 0.3mg/ml ascorbic acid (Sigma) was added to apomorphine to retard oxidation. All drug solutions were made fresh each day and coded prior to using.

Apparatus

Tonic immobility

Tonic immobility was measured on the floor of a small BRS sound-attenuated chamber (SEC-002) with the door open for visual observation.

Locomotor activity

Four activity platforms (BRS Foringer Model JPA-001) which provided pulses when a plumb swung 1mm in any direction as a consequence of the platform moving in the horizontal plane, were used. A white aluminium box (22 x 24 x 28cm), with clear perspex top and front, and a grid floor, was placed on each platform to hold the animals during testing. Two platforms were placed in a large BRS sound-attenuated and vented chamber (LEC-001), separated by an aluminium wall placed midway in the chamber. In each half of the chamber was a light and a speaker. Masking noise (approximately 70 dB) originating from a BRS Foringer audio generator (Model AU 902) was transmitted through the speakers during all behavioural sessions. Printing counters converted from Royal 310PD printing calculators recorded counts cumulated over periods of 20min. Logic and timing was controlled by Digital M-logic modules mounted in a Digital H916 panel and power supply.

Procedure

Haloperidol tonic immobility

Thirty-six chickens (approximately five weeks old) were randomly assigned to three groups (0, 0.5 and 1.0mg/kg haloperidol). Thirty mins following injection of the respective dose of haloperidol the animal was placed in the testing chamber and manually restrained on its right side for 15sec. Tonic immobility was measured as the time in sec from when the animal was released by the experimenter until it rose to its feet. Additional induction attempts were given 60sec after each unsuccessful attempt to elicit tonic immobility. If the animal failed to show tonic immobility over five attempts it was given a score of zero sec. The floor was cleaned and wiped with a weak vinegar solution between each test.

Apomorphine activity

Forty chickens (approximately five weeks old) were randomly assigned to four groups (0, 1.0, 2.0 and 4.0 mg/kg apomorphine). The chickens were individually placed on the activity platforms at either 10am or 2pm and their activity recorded for 3 periods of 20min each. They were then injected intraperitoneally with apomorphine and immediately replaced on the activity platform for another three 20min periods.

Haloperidol-apomorphine activity

Twenty chickens (approximately six weeks old) were randomly assigned to two groups. All animals were given one hour of habituation in the activity platform as described above. One group was then injected with haloperidol (1.0mg/kg) and the other group with the vehicle (1.0mg/kg). They were immediately replaced in the activity platform for one 20min period. Both groups were then injected with apomorphine (2.0mg/kg) and immediately replaced for three 20min periods.

Apomorphine tonic immobility

Eighteen chickens (approximately 3 weeks old) were randomly assigned to two groups (0 and 2.0mg/kg apomorphine) and tested as described above (haloperidol tonic immobility).

Scopolamine tonic immobility

Eighteen chickens (approximately four weeks old) were randomly assigned to two groups (0 and 2.0mg/kg scopolamine) and tested as described above (haloperidol tonic immobility).

Scopolamine activity

Forty chickens (approximately three weeks old) were randomly

assigned to four groups (0, 0.5, 1.0 and 2.0mg/kg scopolamine) and tested as described above (apomorphine activity).

Statistics

One-way analyses of variance were used to analyse the habituation and haloperidol tonic immobility data. Two-way analyses of variance were used to analyse all drug-induced activity data. Student's t test was used to analyse the significance of the haloperidol-apomorphine activity and the scopolamine tonic immobility data. Because of heterogeneity of variance, a square root transformation was performed on the data prior to analysis.

RESULTS

Haloperidol tonic immobility

The results shown in Table 1 reveal a significant effect of haloperidol on tonic immobility, compared to controls ($F = 13.26$, $df = 2, 24$, $p < 0.01$).

Apomorphine activity

The results are shown in Fig. 1. Before apomorphine administration there were no significant differences in motor activity between any of the groups, as shown in the last 20min ($F < 1$). Injection of apomorphine, however, produced a dose dependent increase in activity ($F = 4.17$, $df = 3, 108$, $p < 0.01$), with 2mg/kg producing the maximum stimulation of activity. The progressive decrease of activity over time did not quite reach significance ($F = 2.66$, $df = 2, 108$, $p < 0.08$). There was no significant interaction effect of dose with time ($F = 1.17$, $df = 6, 119$, $p > 0.10$).

Haloperidol-apomorphine activity

The results are shown in Fig. 2. Prior to any drug administration there was no significant difference in motor activity between the two groups as shown in the last 20min ($t < 1$). Similarly, there was no difference between groups for the 20min period following haloperidol

Table 1.

The Effect of Haloperidol on the Duration of Tonic Immobility (sec.)

	Dose (mg/kg)		
	0	0.5	1.0
Mean	190.9	720.3	1480.4
Standard Error	45.4	131.5	363.3

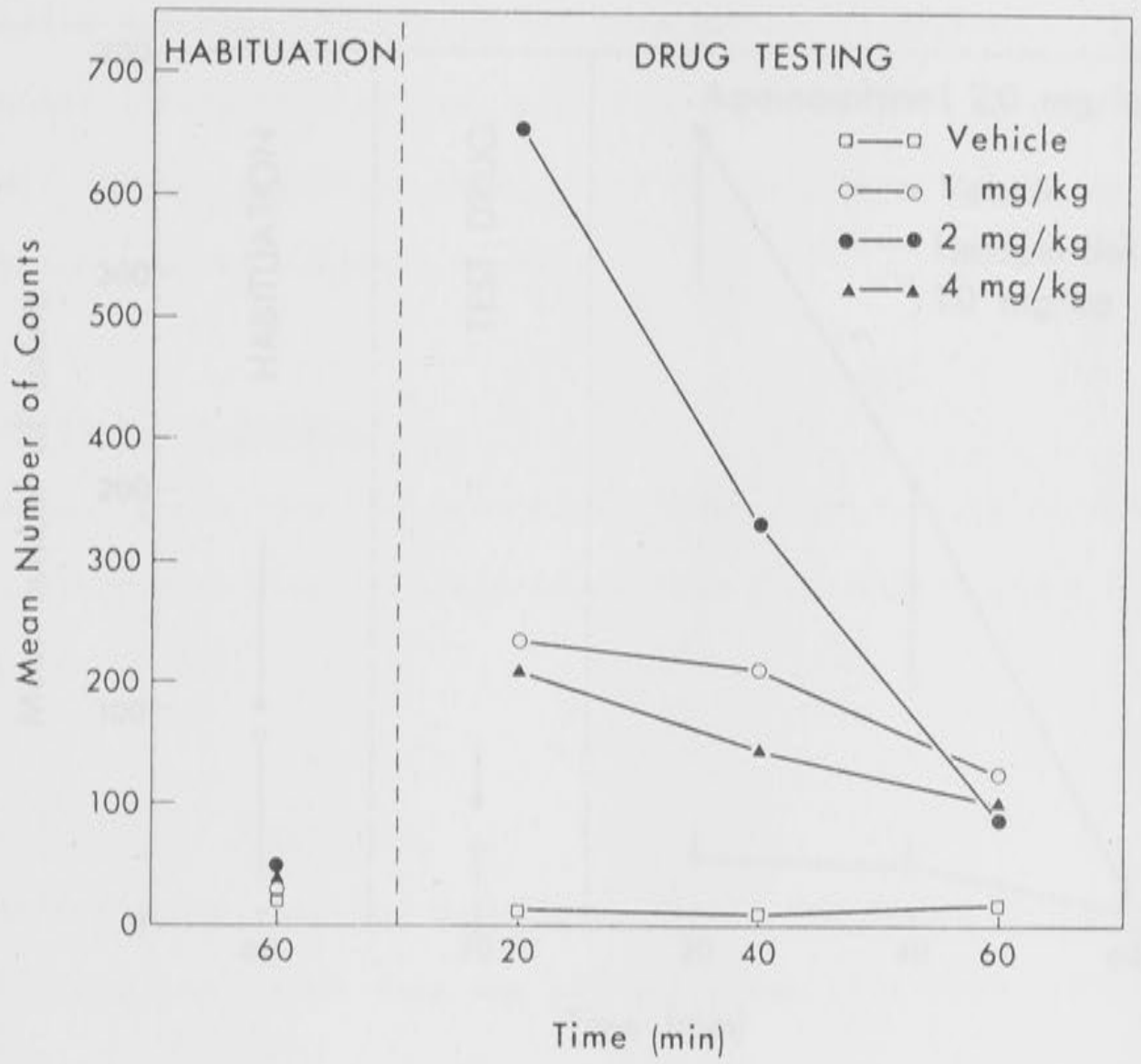

Fig. 1. The effect of varying doses of apomorphine on motor activity of chickens before (left panel) and after (right panel) drug administration. Habituation includes the results of the last 20min in a 60min test.

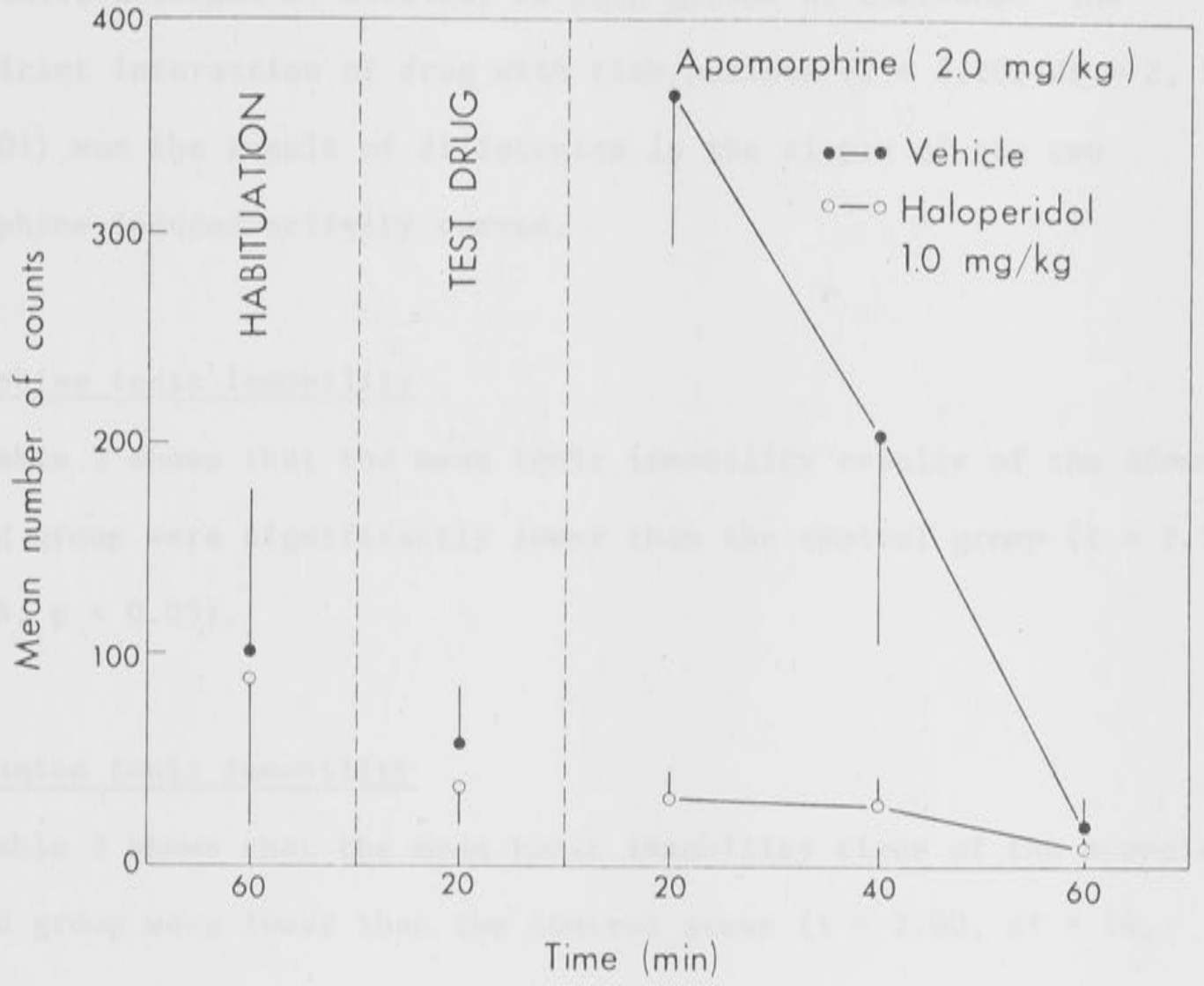

Fig. 2. The effect of haloperidol (test drug) on apomorphine-induced motor activity. Habituation includes the results of the last 20min in a 60min pre-drug activity test. Error bars are standard errors of the means.

injection ($t < 1$). However, following apomorphine administration, motor activity was not as markedly increased in the haloperidol pre-treated group, compared to controls ($F = 17.74$, $df = 1, 54$, $p < 0.001$). The significant effect of time periods ($F = 6.91$, $df = 2, 54$, $p < 0.005$) reflected the progressive decrease of activity of both groups of chickens. The significant interaction of drug with time periods ($F = 5.20$, $df = 2, 59$, $p < 0.01$) was the result of differences in the slopes of the two apomorphine-induced activity curves.

Apomorphine tonic immobility

Table 2 shows that the mean tonic immobility results of the apomorphine treated group were significantly lower than the control group ($t = 2.14$, $df = 16$, $p < 0.05$).

Scopolamine tonic immobility

Table 3 shows that the mean tonic immobility times of the scopolamine treated group were lower than the control group ($t = 2.00$, $df = 16$, $p < 0.06$).

Scopolamine activity

The results are shown in Fig. 3. Before scopolamine administration there were no significant differences in motor activity between any of the groups as shown in the last 20min ($F < 1$). After administration of scopolamine, however, there was a significant dose dependent increase in activity ($F = 4.55$, $df = 3, 108$, $p < 0.005$). There were no significant effects of time ($F < 1$) or interaction of dose with time ($F < 1$).

Table 2.

The Effect of Apomorphine on the Duration of Tonic Immobility (sec)

	Dose (mg/kg)	
	0	2.0
Mean	284.6	145.1
Standard Error	48.7	43.2

Table 3.

The Effect of Scopolamine on the Duration of Tonic Immobility (sec)

	Dose (mg/kg)	
	0	2.0
Mean	214.7	70.9
Standard Error	66.6	27.2

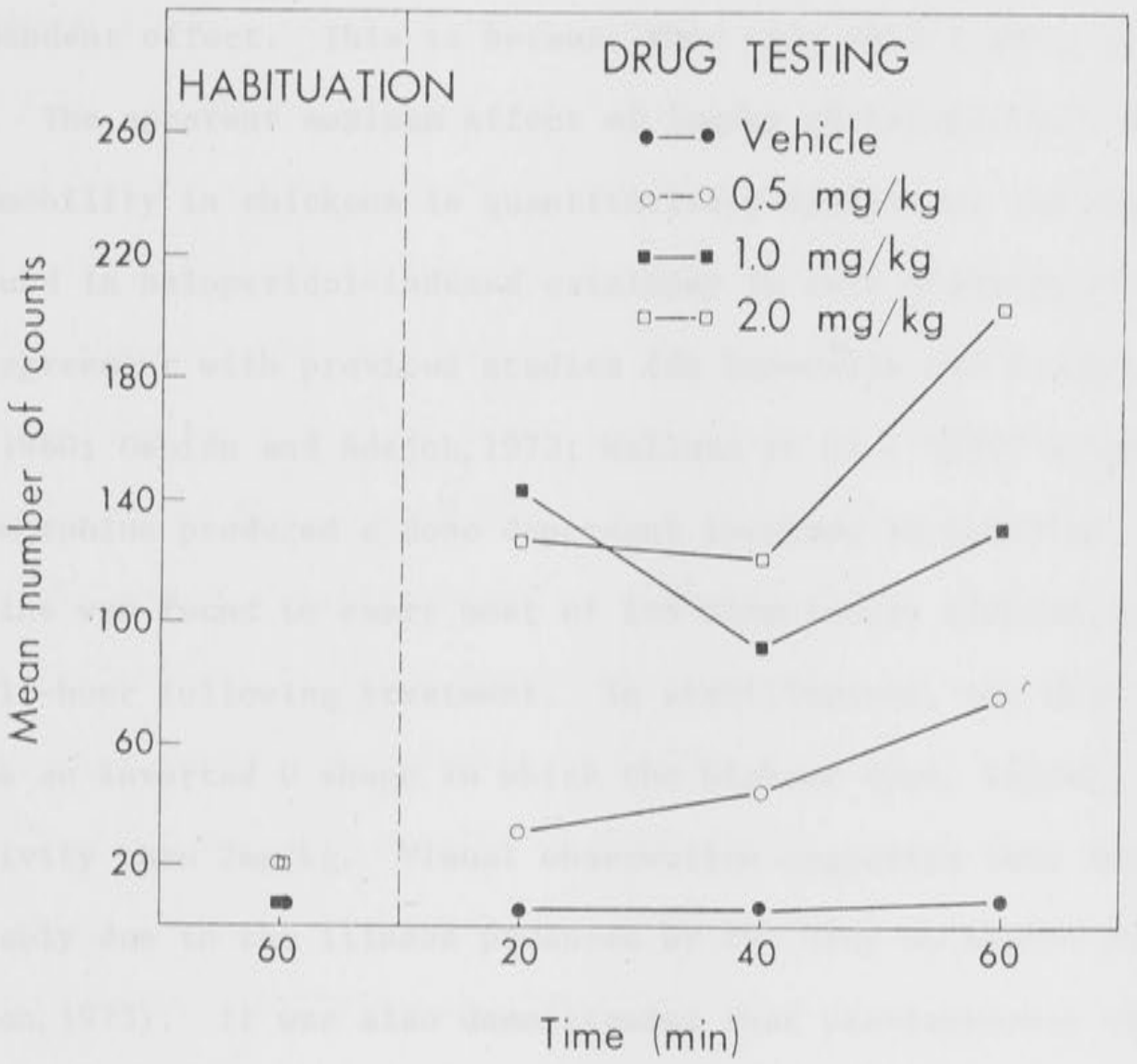

Fig. 3. The effect of varying doses of scopolamine on motor activity of chickens before (left panel) and after (right panel) drug administration. Habituation includes the results of the last 20min in a 60min test.

DISCUSSION

The dopaminergic receptor antagonist, haloperidol, was found to produce a dose dependent increase in tonic immobility duration. This is consistent with findings by Wallnau *et al.* (1979), except that they did not find a dose dependent effect. This is because they only used 1 and 2 mg/kg of haloperidol. The apparent maximum effect of 1mg/kg of haloperidol to potentiate tonic immobility in chickens is quantitatively similar to the dose response curve found in haloperidol-induced catalepsy in rats (Sanberg *et al.*, 1979).

In agreement with previous studies (de Lanerolle and Youngren, 1978; Machlis, 1980; Osuide and Adejoh, 1973; Wallnau *et al.*, 1979) it was found that apomorphine produced a dose dependent increase in activity in chickens. Apomorphine was found to exert most of its stimulatory effects during the first half-hour following treatment. In stabilimeters, the dose response curve was an inverted U shape in which the highest dose, 4mg/kg, produced less activity than 2mg/kg. Visual observation suggested that this effect was probably due to the illness produced by the drug at higher doses (Osuide and Adejoh, 1973). It was also demonstrated that pre-treatment with haloperidol, at a time known to produce a high degree of tonic immobility potentiation (Wallnau *et al.*, 1979), completely antagonized the stabilimeter activity inducing effects of apomorphine. de Lanerolle and Youngren (1978) also showed that haloperidol antagonized apomorphine-induced stereotyped behaviours. These results suggest that in chickens apomorphine and haloperidol are acting at the same dopamine receptor sites, as found in mammals.

The effect of the dopaminergic agonist, apomorphine, was found to reduce the duration of tonic immobility in the present study. This is consistent with previous reports showing that apomorphine (Wallnau *et al.*, 1979) and d-amphetamine (Boren and Gallup, 1976) significantly decreased tonic immobility. Conversely, Ettinger and Thompson (1978) found that a

high dose of l-dopa enhanced the duration of tonic immobility. Since Ettinger and Thompson (1978) did not use a decarboxylase inhibitor in conjunction with l-dopa, it is possible that their results are a function of the peripheral effects of l-dopa.

Thompson *et al.* (1974) first reported that scopolamine reduced tonic immobility in chickens. However, Ksir (1978) later reported that scopolamine had no effect on tonic immobility in chickens, and concluded that there was experimenter bias in the work of Thompson *et al.* (1974). Our results show a large, but not quite significant, decrease in tonic immobility following a dose of scopolamine which produced the maximum effect according to Thompson *et al.* (1974). We have found that different batches of chicks, tested at different times of the year, can show vast differences in tonic immobility behaviour (unpublished data), as indeed does general performance on learning tasks (Anson, 1980). It is possible that the negative results of Ksir (1978) were a function of chickens which normally would have shown relatively large tonic immobility durations (greater than 180sec.). Thus, the incorporation of a cut-off period in his chickens at 180 sec may have led to the scopolamine and control groups showing similar durations.

The increase in stabilimeter activity following scopolamine was similar to that found by Thompson *et al.* (1974) and Ksir (1978) in photocell activity cages. Thompson *et al.* (1974) measured the effects of 0.5mg/kg for 10min, whereas Ksir (1978) used two doses (0.5 and 1.0mg/kg) for 1h of testing. Ksir (1978) found that both doses of scopolamine produced effects over the full hour as was similar to that seen in the present study. In rats, also, scopolamine produces increased activity lasting over an hour (Sanberg *et al.*, 1979). The dose response seen in the present study, with 2.0mg/kg scopolamine producing maximum effects, was similar to the dose response curve of scopolamine on tonic immobility found by Thompson *et al.* (1974).

Although the present study did not investigate the effects of dopaminergic and cholinergic interactions on motor behaviour, Spooner and Winters (1966) have demonstrated that the anticholinergic, atropine, was very effective in potentiating amphetamine effects in young chickens. Thus, it appears that in birds, as in rats, the dopaminergic and cholinergic systems are intimately involved in the expression of motor behaviour. More research is needed to clarify these relationships.

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THE EFFECT OF STRIATAL LESIONS IN THE CHICK ON HALOPERIDOL-POTENTIATED TONIC IMMOBILITY: A PRELIMINARY STUDY.

Unpublished.

ABSTRACT

The potentiating effects of haloperidol on tonic immobility in chickens were reduced in birds given bilateral injections of kainic acid into the paleostriatum. Aspiration of tissue above the lamina medullaris dorsalis, which includes the neostriatum and hyperstriatum, had no effect on haloperidol-potentiated tonic immobility. The results support the suggestion that the paleostriatum of birds is homologous to the basal ganglia of mammals.

The avian telencephalon has only a very small region of clearly laminated pallium. The rest of the hemisphere is generally divided into several "striatal" regions. The striated appearance of these regions is quite similar to the corpus striatum in mammals: and thus the entire avian telencephalon was commonly referred to as the corpus striatum (Ariens Kappers *et al.*, 1936). However, more recent anatomical and histochemical studies have shown that only the basal part of the telencephalon, the paleostriatum, may be truly homologous to the mammalian corpus striatum. The regions above the lamina medullaris dorsalis, including mainly the neostriatum and hyperstriatum, are considered to be homologous to the mammalian neocortex. The paleostriatal complex is divided into the paleostriatum augmentatum, the paleostriatum primitivum and within this structure, the nucleus intrapeduncularis. Available evidence suggests that the paleostriatum augmentatum is homologous to the neostriatum of mammals, whereas the paleostriatum primitivum and nucleus intrapeduncularis represent the avain counterparts of the lateral and medial divisions of the globus pallidus (Brauth *et al.*, 1978; Karten and Dubbeldam, 1973).

As in the mammalian striatum, the paleostriatum augmentatum contains the highest concentration of dopamine, which probably originates from the nucleus tegmenti pedunculopontis via the tegmentopaleostriatal tract (homology of the mammalian nigrostriatal tract) (Nistico' and Stephenson, 1979). The paleostriatum augmentatum also receives a topographical projection from the neostriatum, which is considered to be homologous to the mammalian corticostriatal pathway (Brauth *et al.*, 1978; Karten and Dubbeldam, 1973).

While this view of the anatomical homologies is generally accepted, there has been little work showing a functional homology between the avian neostriatum and paleostriatum, and the mammalian neocortex and basal ganglia. In mammals the striatum is involved in the expression of

motor behaviour, controlled in part by dopaminergic mechanisms (Dray, 1979). Birds also exhibit motor responses, similar to mammals, following administration of various drugs affecting dopaminergic transmission (Nistico' and Stephenson, 1979). These dopaminergic influences on motor activity appear to be mediated by the paleostriatum augmentatum (Nistico' and Stephenson, 1979). Goodman and his colleagues (1977, 1978) demonstrated that lesions of the paleostriatum attenuate apomorphine-induced stereotyped behaviours, whereas lesions of the dopaminergic tegmentopaleostriatal pathway potentiated apomorphine effects, probably due to the supersensitivity of the dopamine receptors on paleostriatal neurons, which develop as a consequence of deafferentation (Dray, 1979). The present study examined further the possible functional homologies, by determining in chickens the effects of lesions of these areas on tonic immobility potentiated by the dopamine receptor antagonist haloperidol (Wallnau *et al.*, 1979). In rats, haloperidol-induced catalepsy is mediated by dopamine receptors localized on striatal neurons (Sanberg, 1980). Thus lesions of the striatum attenuated, whereas cortical lesions did not affect haloperidol-induced catalepsy. Catalepsy in rats appears similar to tonic immobility in chickens (Sanberg *et al.*, 1980). It was therefore of interest to determine the effects of lesions of the neostriatal and paleostriatal regions of the chick telencephalon on haloperidol-potentiated tonic immobility in chickens.

One day-old male domestic chicks (crosses between White Leghorn and Black Australorp) were obtained from Research Poultry Farm (Victoria, Australia). They were housed in groups in 23 x 23 x 29cm metal cages with perspex fronts and given free access to chick starter crumble (Hutmill, Victoria) and water (tap water with terramycin added).

On day three of age, nine chicks were partially decerebrated, leaving most of the basal telencephalon intact. In chicks, anaesthetized with halothane and positioned on a Kopf stereotaxic instrument, the skin

and calvaria overlying the forebrain was reflected and brain tissue was removed by aspiration to the level of the lamina medullaris dorsalis, which forms the dorsal and lateral boundaries of the paleostriatal complex. Bleeding was controlled by gelfoam. Nine control chicks received sham operations in which they underwent the same surgery except no tissue was aspirated. In addition to the paleostriatum, tissue left behind included the parts of the basal telencephalon medial and basal to the paleostriatum, i.e., the paraolfactory lobe, olfactory tubercle, septal and preoptic regions. Another ten chicks received bilateral injections of kainic acid into the paleostriatum. The chicks were anaesthetized with halothane and held in a Kopf stereotaxic instrument. 3nmole of kainic acid in 0.3µl of saline was injected at the following coordinates according to the Youngren and Phillips atlas (1978); AP = + 3.8, ML = $\pm$ 2.4, DV = + 5.8. The kainic acid was injected via a 30 gauge Hamilton syringe at a rate of 0.3µl/min. After injection the cannula was left in place for a further 2min to allow diffusion of the drug solution. Rieke (1980) and preliminary observations show that these injections destroy paleostriatum augmentatum, paleostriatum privitum and nucleus intrapeduncularis neurons. All the chicks received a prophylactic subcutaneous injection of aquacaine (0.1ml) to prevent infection.

Four weeks later the effect of haloperidol (McNeill) on tonic immobility was assessed in the birds. Tonic immobility was measured on the floor of a small BRS sound-attenuated chamber with the door open for visual observation. The birds in each group were randomly assigned to three groups and received i.p. injections of either 0, 0.5, or 1.0mg/kg haloperidol in distilled water. Thirty min. after injection, the animal was placed in the testing chamber and manually restrained on its right side for 15sec. Tonic immobility was measured as the time in sec. from when the animal was released by the experimenter to when it rose to its feet.

Additional induction attempts were given 60sec after each unsuccessful attempt to elicit tonic immobility. If the animal failed to show tonic immobility over five attempts it was given a score of zero sec. The floor was cleaned and wiped with a weak vinegar solution between each test. Tonic immobility was again assessed in the birds two more times (separated by at least 3 drug-free days) and, in a randomly ordered manner, they then received the dose of haloperidol they had not previously received. Two-way analyses of variance were used to evaluate the statistical significance of the data.

The results shown in Fig. 1 show no differences between partially decerebrated chicks and controls on tonic immobility following either 0, 0.5 or 1.0mg/kg haloperidol ($F < 1$). The significant effect of dose ($F = 12.38$, $df = 2, 48$, $p < 0.001$) reflected the dose dependent increase in tonic immobility. In the kainic acid-injected birds there was a significant group difference ($F = 12.49$, $df = 1, 54$, $p < 0.001$), compared to controls (Fig. 2). This reflected the lower response of the kainic acid-injected group to the potentiating effects of haloperidol on tonic immobility. There was also a significant dose effect in this study ($F = 14.00$, $df = 2, 54$, $p < 0.001$). The fact that the interaction of dose with groups was not significant ($F = 2.60$, $df = 2, 59$, $p < 0.05$) meant that both groups showed a dose-dependent increase in the duration of tonic immobility.

The present finding that bilateral paleostriatal lesions reduced the effects of the dopamine receptor antagonist, haloperidol, adds to the work of Goodman and Stitzel (1977). They showed that the effects of the dopamine receptor agonist, apomorphine, are decreased in pigeons given electrolytic lesions of the paleostriatum.

Removal of the neostriatal and hyperstriatal areas above the lamina medullaris dorsalis had no effect on haloperidol's ability to potentiate tonic immobility in chickens. However, kainic acid injections

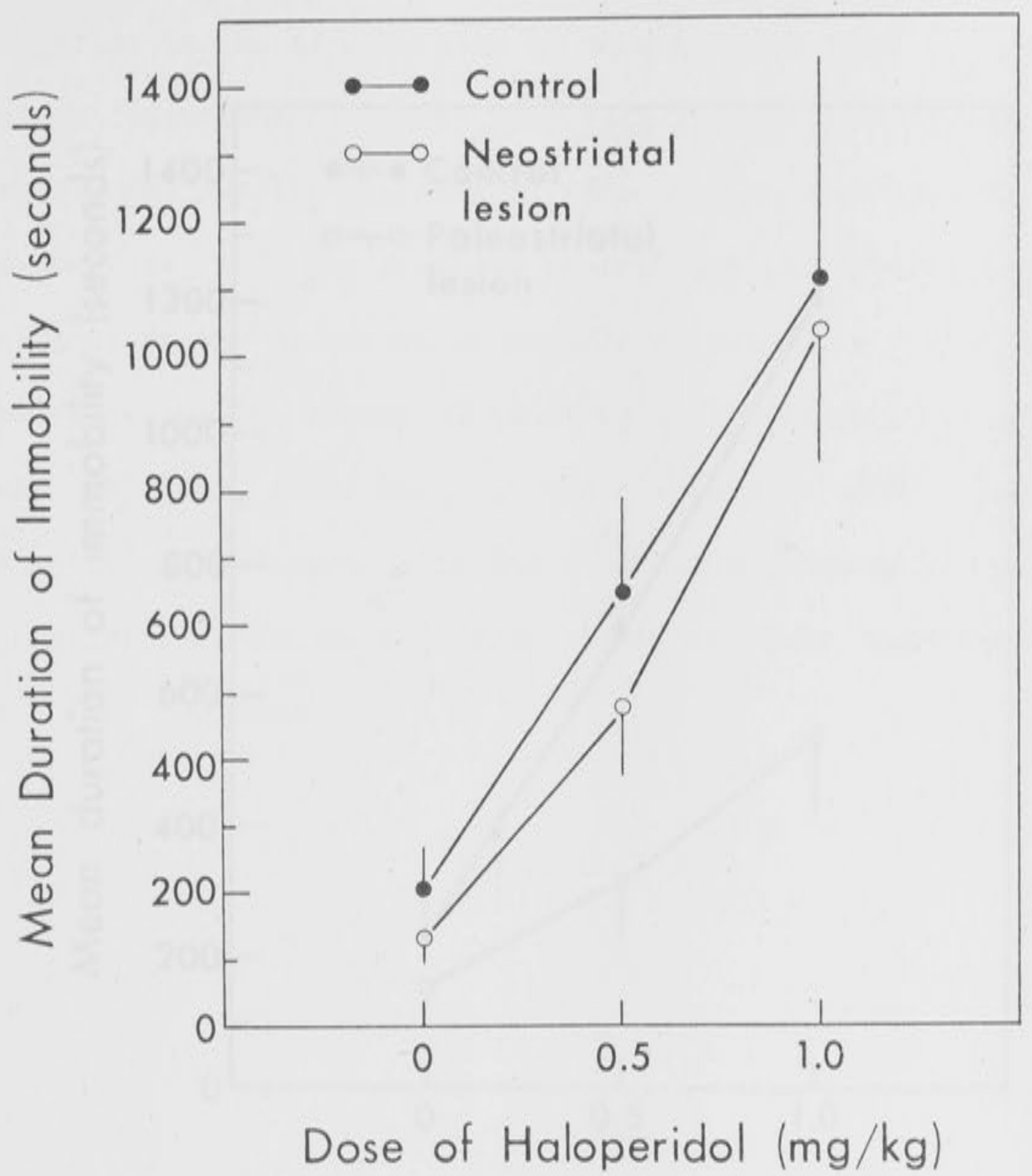

Figure 1. The effect of varying doses of haloperidol on the duration of tonic immobility in control (n = 9) and neostriatally lesioned (n = 9) chickens. Error bars represent standard errors of the means.

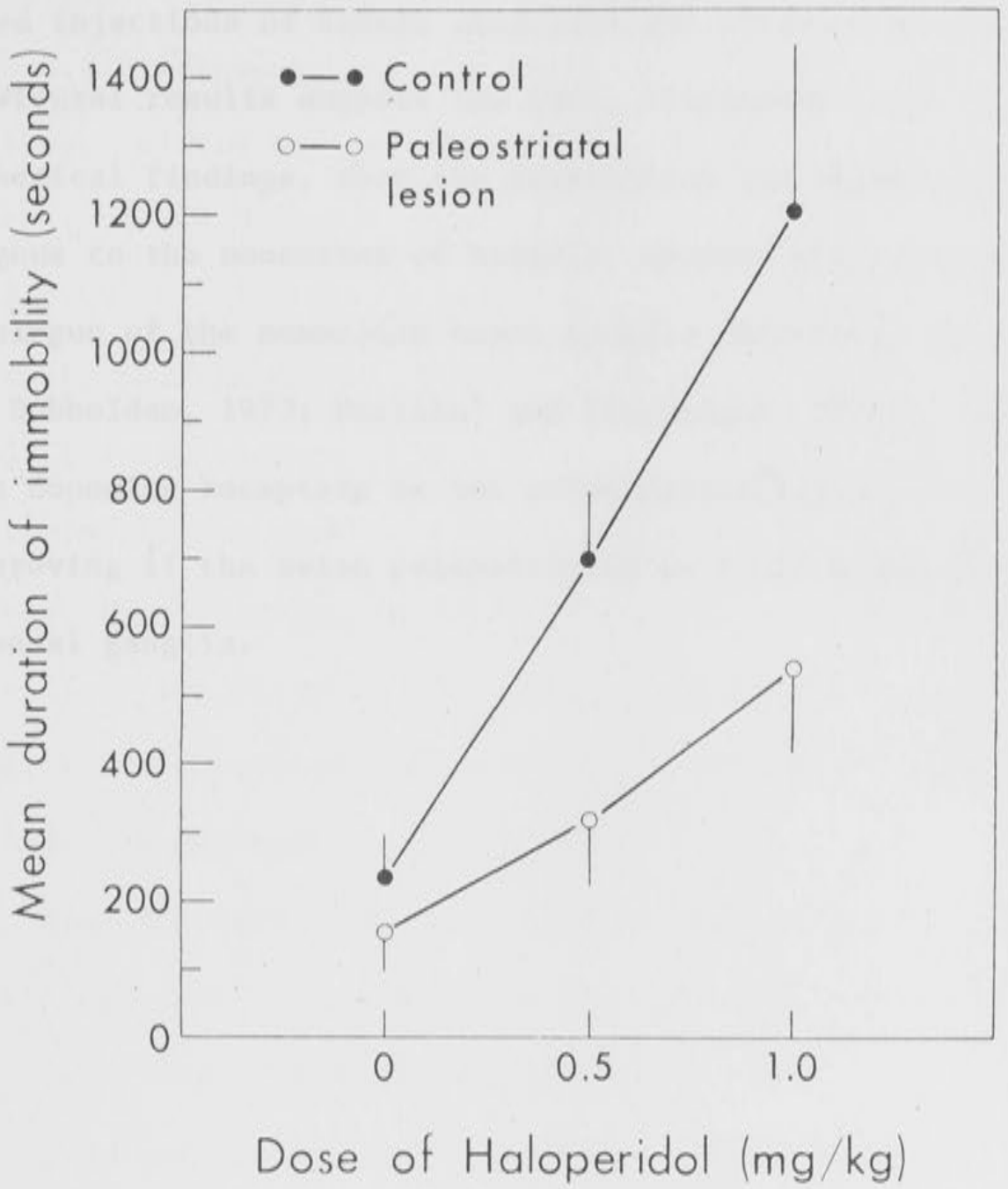

Figure 2. The effect of varying doses of haloperidol on the duration of tonic immobility in control (n = 10) and paleostriatally lesioned (n = 10) chickens. Error bars represent standard errors of the means.

into the paleostriatum decreased the effectiveness of haloperidol's action. This is similar to the effects seen in decorticated rats, or those which had received injections of kainic acid into the striatum (Sanberg, 1980). These behavioural results support the idea, originally based on anatomical and histochemical findings, that the neostriatum and hyperstriatum in birds are homologous to the neocortex of mammals, whereas the avian paleostriatum is the homologue of the mammalian basal ganglia (Brauth *et al.*, 1978; Karten and Dubbeldam, 1973; Nistico' and Stephenson, 1979). Continued research on dopamine receptors in the avian paleostriatum should be useful in proving if the avian paleostriatum is truly homologous to the mammalian basal ganglia.

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PERMANENT EFFECTS OF INJECTIONS OF CYCLOHEXIMIDE OR GLUTAMATE IN THE CHICK FOREBRAIN ON MOTOR ACTIVITY INDUCED BY APOMORPHINE OR SCOPOLAMINE.

Unpublished.

ABSTRACT

Compared to controls, bilateral forebrain injections of cycloheximide or glutamate in 2 day-old chicks resulted in significantly less activity in response to intraperitoneal injections of the dopaminergic agonist, apomorphine, or the cholinergic antagonist, scopolamine, when tested on stabilimeters four weeks later. The results are consistent with the idea that neonatal injections of cycloheximide or glutamate cause damage to neurotransmitter mechanisms localized on intrinsic and/or efferent neurons in the paleostriatal complex (basal ganglia), the nuclei responsible for motor behaviour in birds.

Key words: Cycloheximide, glutamate, motor activity, apomorphine, scopolamine, paleostriatum.

Previous studies have demonstrated profound behavioural alterations in older chickens which received forebrain injections of cycloheximide (a ribosomal protein synthesis inhibitor) or glutamate when newly hatched. Initially, Rogers *et al.* (1974) demonstrated that an intracranial injection of cycloheximide would subsequently render the chicken incapable of learning at the normal rate on a pebbled floor task. These chicks failed to reach control levels of performance, by not choosing predominantly grain, in preference to pebbles, in the latter half of the 60 pecks of the test. Rogers and Anson (1978) reported that birds treated with cycloheximide persisted in pecking at preferred food more than saline-injected control animals. In addition, birds treated with cycloheximide were found to be slower to habituate to visual and auditory stimuli (Rogers *et al.*, 1974). Sanberg *et al.* (1981), however, could not find any impairment in the acquisition, maintenance of extinction of an instrumental appetitive key-pecking response by cycloheximide-treated birds. However, these birds were less active and displayed more emotional behaviours than control animals in an open-field. Tonic immobility, a good measure of fear in chickens (Gallup 1979) was also enhanced in cycloheximide-treated birds (Sanberg *et al.*, 1981).

Subsequently, Hambley and Rogers (1979) postulated that cycloheximide produced these long term behavioural changes in chicks as a result of an accumulation of excitatory amino acid neurotransmitters, particularly glutamate, as a consequence of decreased utilization of these amino acids for protein synthesis. Furthermore, it was demonstrated that forebrain injections of glutamate produced behavioural deficits qualitatively similar to those seen in birds treated with cycloheximide (Hambley and Rogers, 1979; Sdraulig *et al.*, 1980; Sanberg *et al.*, 1981). Rogers *et al.* (1974) did not detect any neuronal destruction following cycloheximide treatment, but more extensive histology needs to be carried out before

definite conclusions can be drawn.

In this study, we have examined possible neurotransmitter pathology in the paleostriatum of cycloheximide and glutamate treated chickens using drug-induced motor activity techniques (Sanberg and Mark 1981b). A preliminary report has been given elsewhere (Sanberg and Mark 1981a).

METHODS

White Leghorn x Black Australorp male chickens were obtained from Research Poultry Farm (Victoria) on day 1 of life. On day 2 of life, the chicks were randomly assigned to three groups and given bilateral intracerebral injections of either cycloheximide (20µg/25µl vehicle/hemisphere), glutamate (210µg/25µl vehicle/hemisphere) or saline vehicle into the forebrain as previously described (Sanberg *et al.*, 1981). They were housed in randomly mixed groups until one week before testing, at which time they were housed individually in 23 x 23 x 29cm metal cages with a perspex front and given *ad lib* food (chicken crumble, Hutmill, Victoria) and water (tap water with teramycin added).

When five to seven weeks of age, 13 cycloheximide, 13 glutamate and 13 saline injected birds were tested for motor activity on stabilimeters. Four activity platforms (BRS Foringer Model JPA-001) which provided pulses when a plumb swinged 1mm in any direction as a consequence of the platform moving in the horizontal plane were used. A white aluminium box (22 x 24 x 28cm) with clear perspex top and front, and a grid floor was placed on each platform for holding the animals during testing. Two platforms were placed in a large BRS sound-attenuated and vented chamber (LEC-001) separated by an aluminium wall placed midway in the chamber. In each half of the chamber was a light and speaker in which masking noise (approximately 70dB) was delivered. Printing counters recorded counts cumulated over periods of 20min.

The chickens were individually placed on the activity platforms at either 10am or 2pm and their activity recorded for six periods of 20min each. They were then injected intraperitoneally with 2mg/kg apomorphine hydrochloride (Sigma) dissolved in distilled water (1ml/kg) with 0.3mg/ml ascorbic acid (Sigma) added to retard oxidation. Immediately following injection they were replaced on the activity platform for another three 20min periods. Another 33 chickens (11 in each experimental group) were tested for motor activity as described above, except that a one-hour pre-drug testing period was used and the drug employed was 2mg/kg scopolamine hydrobromide (Sigma) dissolved in distilled water.

One and two-way analyses of variance were used to test the statistical significance of the data. Because of skewness and heterogeneity of variance, a square root transformation of data was performed prior to analysis and the median results of the drug-induced activity tests are shown. Because of heterogeneity of variance, Mann-Whitney U tests were performed on the actual increased motor activity over baseline data (Fig. 4).

RESULTS

The mean results of the two-hour non-drug activity test are shown in Fig. 1. Analysis of variance revealed a significant difference between groups ($F = 20.81$, $df = 2, 216$, $p < 0.001$), with the saline treated group showing the most activity. There were no significant effects of time periods ($F < 1$) or interaction of experimental group with time ($F < 1$). Following injection of apomorphine (Fig. 2), the groups treated with cycloheximide and glutamate showed significantly less stimulated activity than the saline treated group ($F = 5.20$, $df = 2, 108$, $p < 0.01$). The significant effect of time periods ($F = 9.98$, $df = 2, 108$, $p < 0.001$) reflected the progressive decrease of activity of the three groups of chickens. There was no significant interaction of experimental group with time ($F < 1$). In the animals that received scopolamine (Fig. 3) there was also a significant effect of groups ($F = 9.36$, $df = 2, 90$, $p < 0.001$),

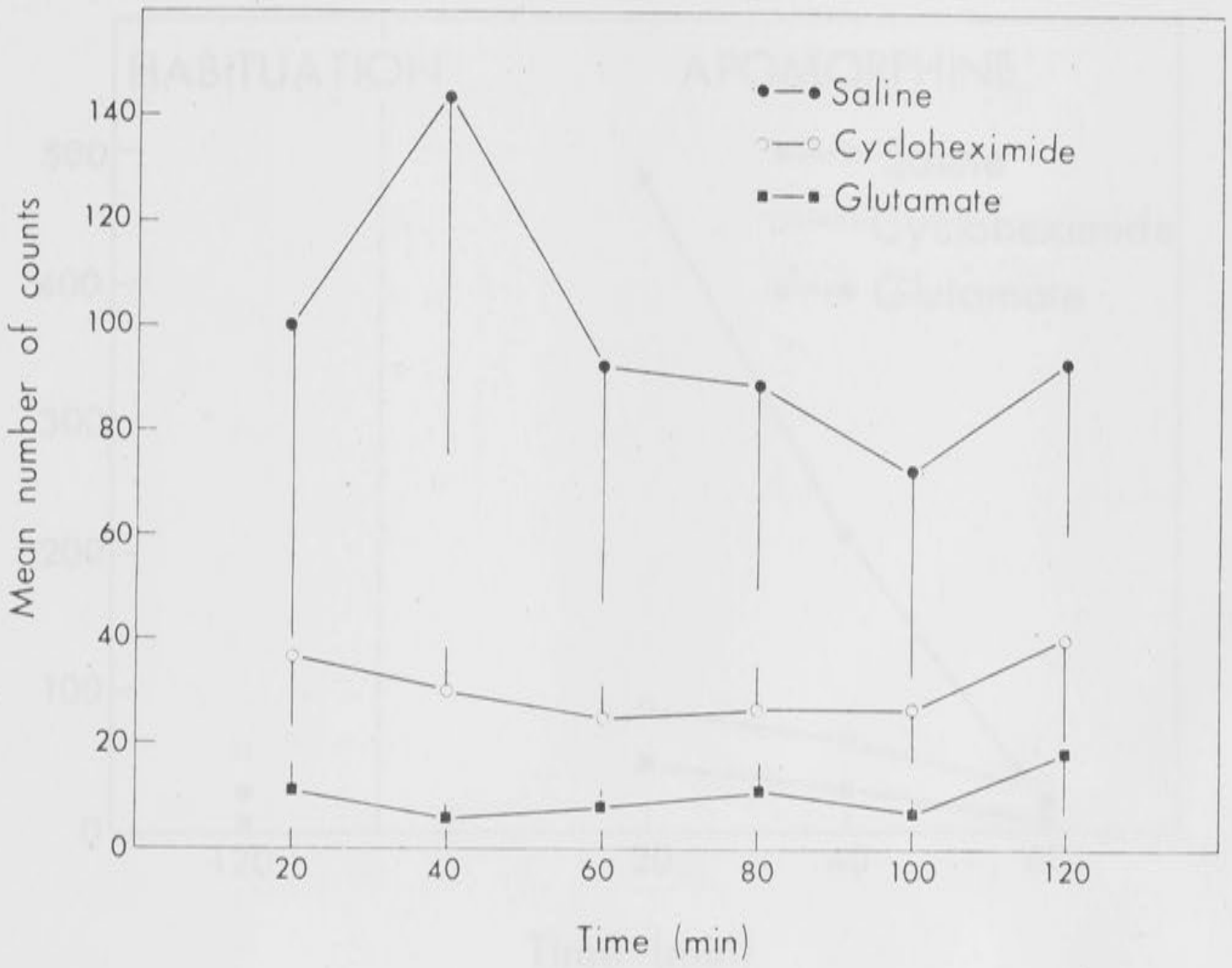

Fig. 1. Spontaneous motor activity of chickens treated with cycloheximide or glutamate. Error bars represent standard errors.

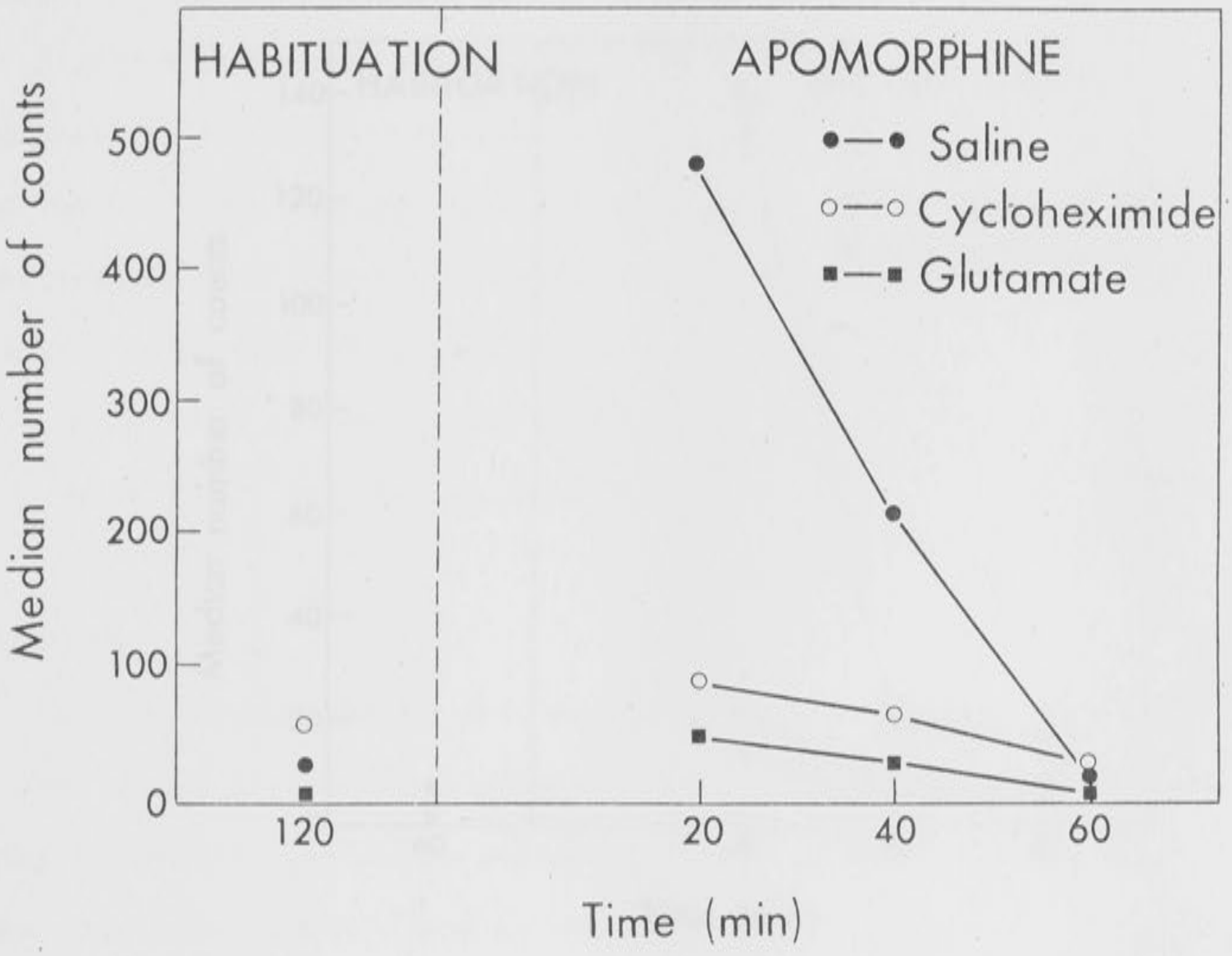

Fig. 2. Median motor activity of chickens treated with cycloheximide or glutamate before (left panel) and after (right panel) administration of apomorphine (2mg/kg). Habituation represents the last 20min of a 120min activity test.

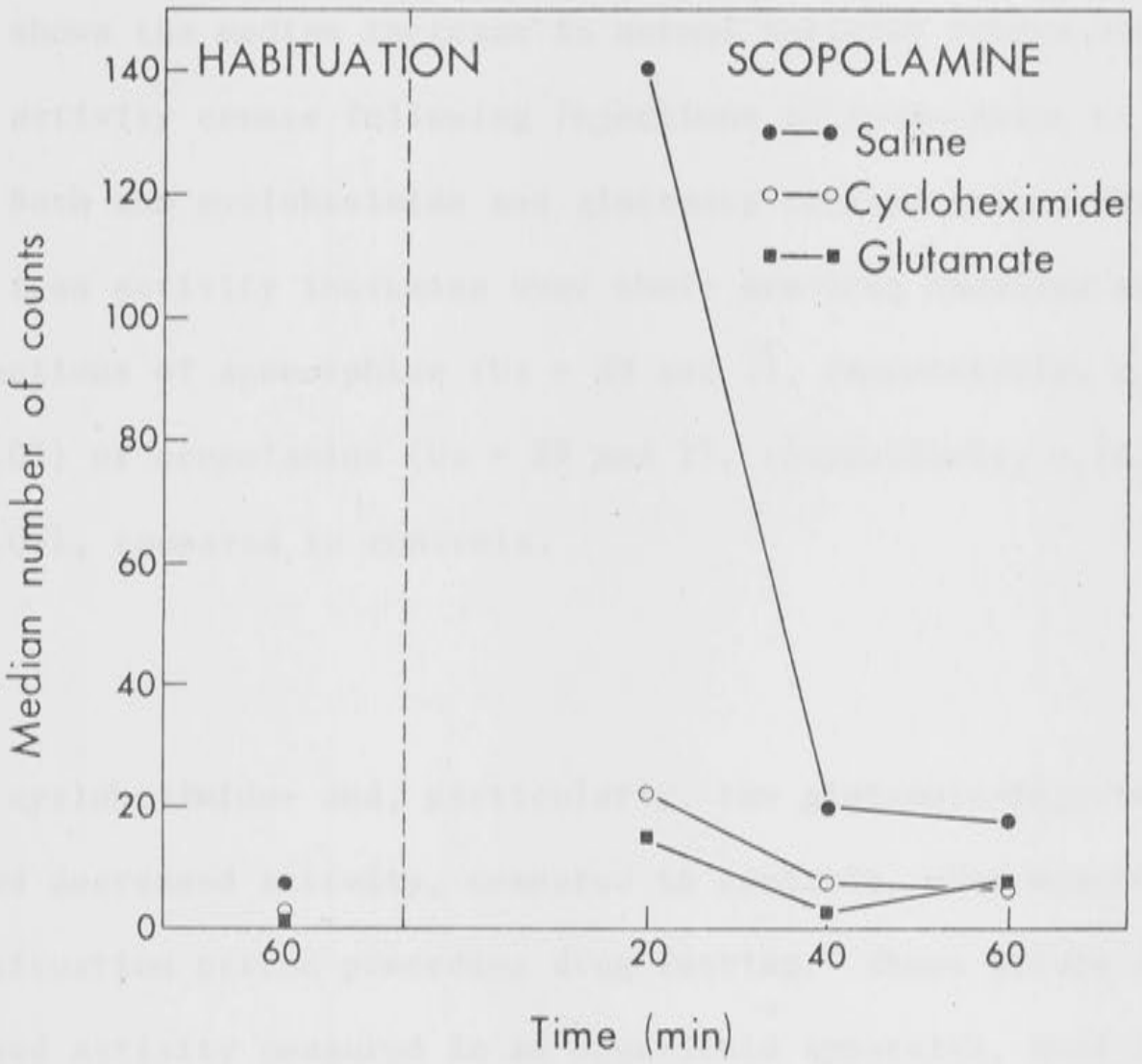

Fig. 3. Median motor activity of chickens treated with cycloheximide or glutamate before (left panel) and after (right panel) administration of scopolamine (2mg/kg). Habituation represents the last 20min of a 60min activity test.

reflecting the greater activity levels in the saline treated group. There were no significant effects of time periods ($F = 2.76$, $df = 2, 90$, $p > 0.05$) or interaction of experimental group with time ($F < 1$) in the scopolamine study.

Figure 4 shows the median increase in actual activity counts over pre-drug baseline activity counts following injections of apomorphine or scopolamine. Both the cycloheximide and glutamate treated groups showed significantly less activity increases over their pre-drug baseline activity following injections of apomorphine (Us = 38 and 37, respectively, n_1/n_2 = 13/13, ps < 0.05) or scopolamine (Us = 29 and 27, respectively, n_1/n_2 = 11/11, ps < 0.05), compared to controls.

DISCUSSION

Both the cycloheximide- and, particularly, the glutamate-injected chickens showed decreased activity, compared to controls, when examined during the habituation period preceding drug testing. These groups also showed decreased activity measured in an open-field apparatus, most likely as a consequence of enhanced emotional reactions (Sanberg *et al.*, 1981). Following injections of either apomorphine or scopolamine, the saline-injected control animals increased their activity in a similar manner to that shown previously (Sanberg and Mark, 1981b). However, the groups given cycloheximide or glutamate exhibited much less of an activity increase after apomorphine or scopolamine injections. It is possible that since their spontaneous activity was also lower than controls, the actual increase in activity following apomorphine or scopolamine may be similar to control values. This was not the case since both groups treated with cycloheximide and glutamate showed a much lower activity increase over pre-drug levels compared to control animals. An explanation in terms of the cycloheximide or glutamate-treated birds reacting more strongly to an injection *per se*

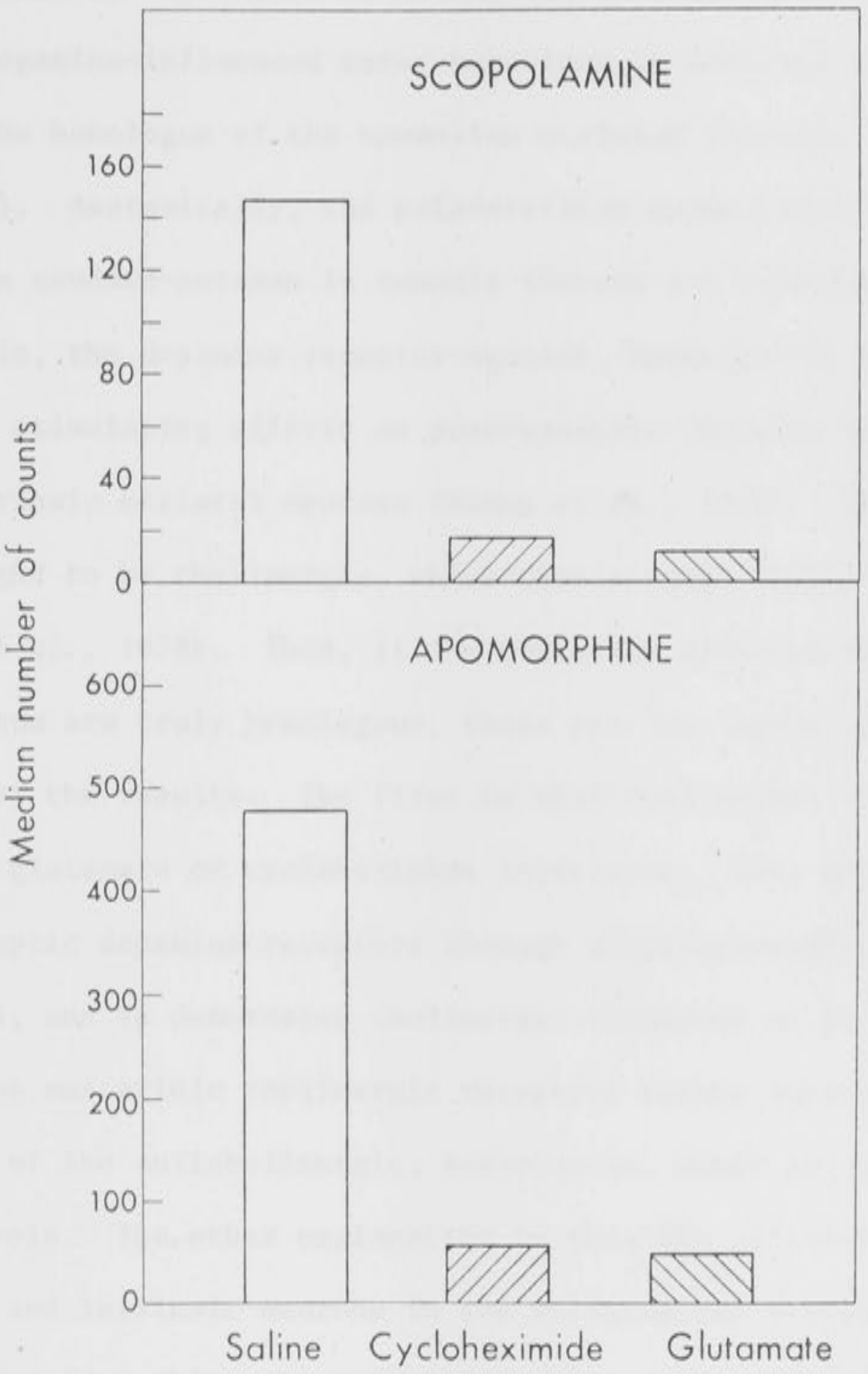

Fig. 4. Median increase in actual motor activity over pre-drug baseline activity in chickens treated with cycloheximide or glutamate following administration of scopolamine (upper panel) or apomorphine (lower panel). Data represents the first 20min of 60min activity test.

is unlikely, since no differences were found between these animals and controls in response to injections of saline (unpublished data).

In birds, dopamine-influenced motor behaviour is mediated by the paleostriatum, the homologue of the mammalian striatum (Nistico' and Stephenson, 1979). Anatomically, the paleostriatum augmentatum is homologous to the caudate-putamen in mammals (Karten and Dubbeldam, 1973). In mammals, the dopamine receptor agonist, apomorphine, probably exerts its motor stimulating effects on post-synaptic dopamine receptors localised on intrinsic striatal neurons (Mason *et al.*, 1978). These neurons are thought to be cholinergic, which then synapse onto efferent neurons (Coyle *et al.*, 1978). Thus, if the mammalian striatum and the avian paleostriatum are truly homologous, there are two likely anatomical interpretations of the results. The first is that cholinergic interneurons are destroyed by glutamate or cycloheximide injections. This would result in less post-synaptic dopamine receptors through which apomorphine could exert its effects, and in denervated cholinergic receptors on efferent neurons. If these muscarinic cholinergic receptors became supersensitive then the effects of the anticholinergic, scopolamine, would be decreased, compared to controls. The other explanation is that the efferent neurons or both efferent and intrinsic neurons in the striatum are decreased, thus reducing any output from this structure induced by dopaminergic or cholinergic mechanisms. The latter explanation seems more plausible, since the former requires that these gross injections of relatively large amounts of glutamate or cycloheximide are more specific than localised intrastriatal injections of glutamate, which destroy both intrinsic and efferent neurons (Coyle *et al.*, 1978).

In birds, these anatomical interpretations are only speculative until further work on the homology between basal ganglia areas of birds and mammals is performed; as well as a positive identification of paleostriatal

neuronal destruction in cycloheximide and glutamate-treated birds is found. Nevertheless, the results suggest that cycloheximide and glutamate injections are causing permanent alterations in dopaminergic and cholinergic neurotransmitter mechanisms, which may be associated with paleostriatal neurons. The paleostriatum augmentation, specifically, shows an intensely large amount of both acetylcholinesterase and dopamine (Karten 1969). Changes in these transmitter systems may explain some of the behavioural abnormalities previously described (Hambley and Rogers 1979; Rogers and Anson 1978; Rogers *et al.*, 1974; Sanberg *et al.*, 1981; Sdraulig *et al.*, 1980).

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CHAPTER THREE

THE STRIATUM AND PSYCHOLOGICAL PROCESSES

Chapter 3.

The Striatum and Psychological Processes

This chapter consists of the following parts and appendices:

Part 3.1 Chapter overview.

Part 3.2.1 Operant, open-field and tonic immobility behaviours in chickens with forebrain injections of cycloheximide or glutamate

Part 3.2.2 Metrazol-induced convulsive thresholds are reduced in chickens with neonatal injections of cycloheximide or glutamate.

Appendix 4 Locomotor activity, exploration and spatial alternation learning in rats with striatal injections of kainic acid.

Appendix 5 Striatal injections of kainic acid selectively impair serial memory performance in the rat.

Appendix 6 Measuring feeding responses in operant research.

Appendix 7 Digital counters: inexpensive alternatives.

Appendix 8 Relationship between tonic immobility and operant conditioning in chickens *Gallus gallus*.

Appendix 9 Relationship of some open-field behaviours to amygdaloid kindled convulsions in Wistar rats.

Appendix 10 Spontaneously recurrent seizures after intracerebral injections of kainic acid in the rat: a possible model of human temporal lobe epilepsy.

Part 3.1 Chapter overview

Many early studies reported psychological impairments in rats with coagulative lesions of the striatum (see Table 1). These methods of lesioning, however, destroy neurons projecting through or terminating in the striatum, thus complicating the interpretation of the behavioural results. The first work on psychological deficits in rats with kainic acid striatal lesions was performed by Sanberg (1978) and published in abstract (Sanberg *et al.*, 1977) and report (Sanberg *et al.*, 1978, 1979) form. Kainic acid lesions circumvented most of the problems associated with electrolytic techniques insofar as only striatal perikarya are destroyed, making it more likely that the psychological deficits were a result of the striatal lesions *per se*.

Rats which received intrastriatal injections of KA were found to be impaired in acquisition and retention of a passive avoidance task (Sanberg *et al.*, 1978). We suggested that rats with striatal lesions have associative disorders which may be similar in mechanism to the cognitive disabilities seen in patients with Huntington's disease, which is characterized, in part, by striatal degeneration. Subsequent analysis showed that the lesioned animals were not different from controls in acquisition of an appetitive bar-pressing response. During extinction of the response, though, they were found to continue responding longer than controls (Sanberg *et al.*, 1979). This suggested that an exaggerated arousal reaction either to lack of expected reinforcement or to occurrence of aversive stimuli

Table 1.

Psychological Tasks in Which Rats with Coagulative Striatal Lesions are Impaired.

Task	References
Avoidance Behaviour	
Passive avoidance	Green *et al.* (1967) Kirkby and Kimble (1968) Mitcham and Thomas (1972) Prado-Alcalá *et al.* (1975, 1979) Rothman and Glick (1976) Winocur (1974) Winocur and Mills (1969)
Active avoidance	Albert and Bignami (1968) Green *et al.* (1967) Hart *et al.* (1978) Kirkby (1970) Kirkby and Kimble (1968) Kirkby and Polgar (1974, 1976) Mitcham and Thomas (1972) Studelska and Beatty (1978) Thomas and Hill (1973) Winocur (1974, 1975) Winocur and Mills (1970)
Sidman avoidance	Allen and Davison (1973) Allen *et al.* (1972)
Operant Behaviour	
CRF	Schmaltz and Isaacson (1972)
FI	Hansing *et al.* (1968)
DRL	Neill *et al.* (1974) Schmaltz and Isaacson (1968)
Sidman avoidance	Allen and Davison (1973) Allen *et al.* (1972)
Alternation	Gross *et al.* (1965)
Maze Behaviour	
Spontaneous alternation	Divac *et al.* (1975) Kirkby (1969)

Spatial alternation	Chorover and Gross (1963) Divac (1971) Hannon and Bader (1974) Hart *et al.* (1978) Kirkby (1969) Mikulas (1966, 1969) Potegal (1969) Schwartzbaum and Donovick (1968)
Delayed Response	Hannon and Bader (1974) Mikulas and Isaacson (1965)
Hebb-Williams Maze	Chorover and Gross (1963) Gross *et al.* (1965) Kirkby (1978a)
Discrimination reversal	Schwartzbaum and Donovick (1968)
Open-field Behaviour	Kirkby (1973, 1975) Studelska and Beatty (1978)
Startle Behaviour	Kirkby (1976)
Exploratory Behaviour	Kirkby (1978b)
Dominance Behaviour	Coyle and Kirkby (1975)
Maternal Behaviour	Kirkby (1967)
Taste Aversion Behaviour	Divac *et al.* (1975)

Abbreviations; CRF, continuous reinforcement; FI, fixed interval; DRL, delayed response learning.

might account at least in part for the abnormally high levels of responding of the striatal lesioned rats after extinction and punishment. Such analysis of the effects of striatal lesions in rats would be consistent in turn with the view that altered arousal levels probably contribute to the cognitive and motor disorders of patients with Huntington's disease (Bruyn, 1968).

Divac *et al.* (1978) have shown that rats taught a delayed, left-right alternation task prior to intrastriatal kainic acid injections were impaired in post-operative retention and re-learning of the task. Two studies in Appendices 4 and 5 (Pisa *et al.*, 1980, 1981) were conducted to further analyse the nature of these behavioural deficits since they indicated a disorder of associative processes. Pisa *et al.* (1980, Appendix 4) found that rats with striatal lesions induced by kainic acid were impaired in food-reinforced spatial alternation but not in spontaneous alternation. Performance of spontaneous alternation did not involve a primary reinforcer and was measured on the basis of fewer daily trials than food-reinforced alternation. It was therefore suggested that either an excessive arousal to appetitive stimuli or an increased susceptibility to interference resulting from accumulated trials, or both, more adequately accounted for the failure in food-reinforced spatial alternation than the alternative interpretations of a general failure of recent memory or a failure of spatial discrimination.

In the study reported by Pisa *et al.* (1981, Appendix 5) rats with striatal lesions induced by kainic acid were trained on a schedule of either singly alternated or continuous reinforcement

in runway apparatus. Both the acquisition and the extinction rates of the kainic acid-treated rats did not significantly differ from those of control rats with either reinforcement schedule. However, the lesioned rats ran significantly more slowly than the controls, especially at the onset of the training sessions, and in contrast to the controls, failed to show reliable speed alternation in the late trials of sessions with reward alternation, thus indicating both a locomotor impairment and an impairment of serial memory performance. These findings, indicating a selective impairment of serial memory performance in a nonspatial task, appear to confirm and to add scope to the interpretations discussed above about the role of the striatum in psychological processes.

The role of the avian 'striatum' in psychological processes is virtually unknown. In the early seventies, Mark and colleagues found that injections of relatively large amounts of cycloheximide into the neostriatal area of the forebrains of newly-hatched chickens produced retarded learning when the birds were tested on a pebble floor task weeks later (Rogers *et al.*, 1974). Cycloheximide is now thought to produce the deficits by increasing the levels of excitatory amino acid transmitters, particularly glutamate and aspartate, in the chick brain as a consequence of decreased protein synthesis (Hambley and Rogers, 1979). Injections of glutamate alone caused similar behavioural deficits (Hambley and Rogers, 1979). The behavioural deficits seen on the pebble floor task were that the chicks injected with cyclo-heximide or glutamate failed to reach the control levels of performance, that is, they failed to choose predominantly grain, in preference to pebbles, in the latter half of the 60 pecks

of the test. Other behavioural deficits found in cycloheximide-injected chicks were slower habituation rates to visual and auditory stimuli (Rogers *et al.*, 1974). In addition, Rogers and Anson (1978) have shown that birds treated with slightly lower doses of cycloheximide persisted in pecking at preferred food more than saline-injected control animals. Because the factors that contribute to performance on the pebble floor are not clearly understood (Reymond and Rogers, 1981a, 1981b) the studies presented in Part 3.2.1 (Sanberg *et al.*, 1981a) were performed in order to elucidate further the behavioural disorders found in chickens injected with cycloheximide or glutamate. In the course of performing these studies, however, new experimental equipment and training procedures for chickens had to be set up. Appendix 6 (Sanberg, 1979) describes a method in which head entries into the food hopper can be measured, giving an indication of feeding responses. Appendix 7 (Sanberg and Bellingham, 1979) shows how calculators can be converted to digital counters inexpensively. Finally, Appendix 8 (Sanberg *et al.*, 1981b) describes the handling and training procedures used to measure acquisition of continuously reinforced appetitive keypecking response in chickens. Fig. 1 shows the laboratory used to carry out the experiments reported in Parts 3.2.1 and 3.2.2.

The experiments reported in Part 3.2.1 showed that chickens that had received bilateral injections of cycloheximide or glutamate into the telencephalon on day 2 of life and tested 4 weeks later showed no deficit in acquisition, performance or extinction of continuously reinforced, appetitive keypecking, as compared to control birds injected with saline.

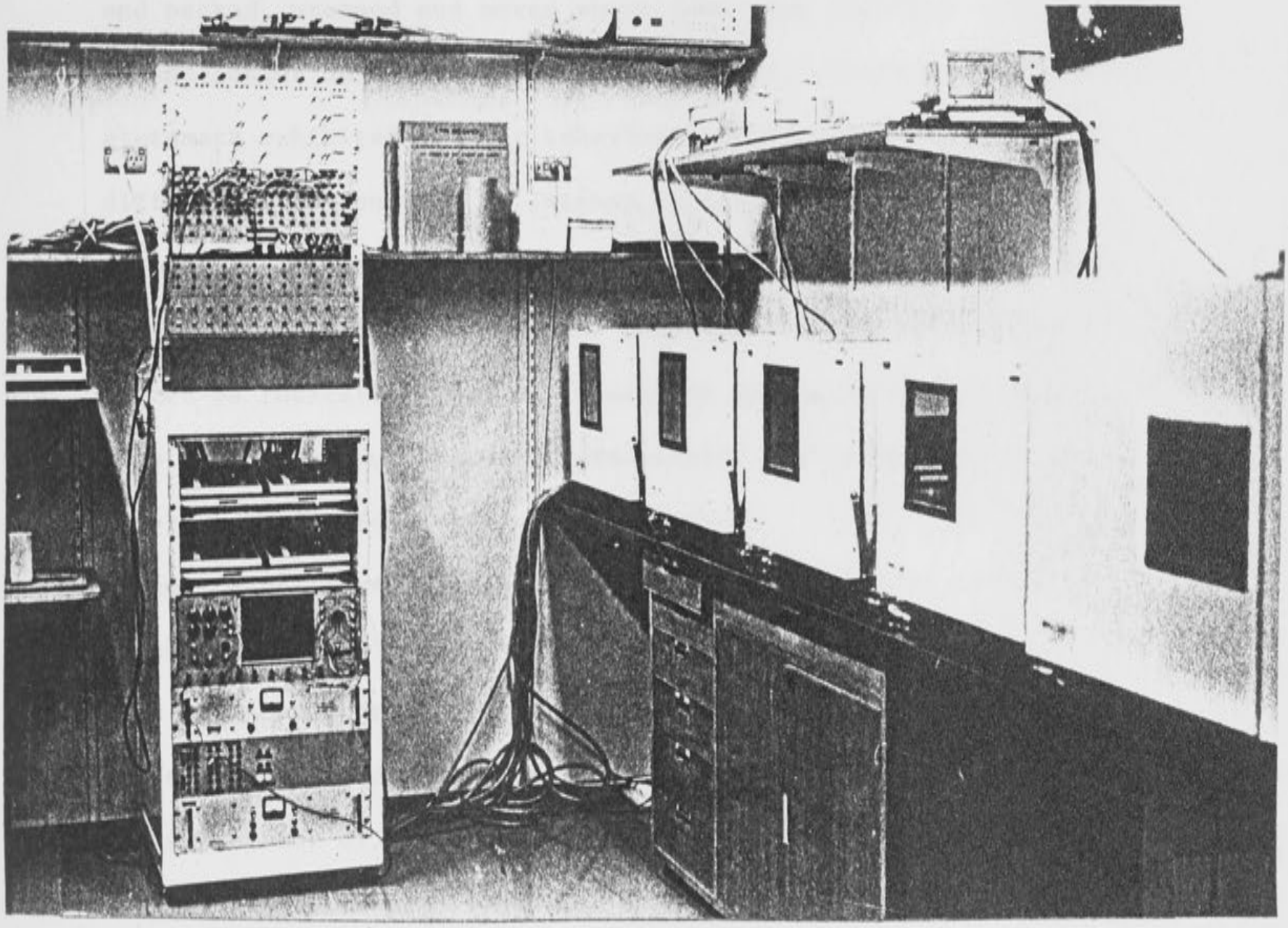

Figure 1. Photograph of laboratory showing four small BRS sound-proof chambers and control panel for programing and data collection.

However, cycloheximide-treated chickens tested in an open-field apparatus took longer to leave the first square, defaecated more, and pecked, preened and moved about less than controls. They also showed longer durations of tonic immobility. Those injected with glutamate exhibited similar behaviour but were not significantly different from controls in latency to leave the first square, in open-field, defaecation or tonic immobility.

Previous studies with the pebble floor task were interpreted as indicating that cycloheximide or glutamate interfered with the neural substrates of associative and memory processes, to produce retarded learning (Rogers *et al.*, 1974). Our results suggest that learning mechanisms *per se* may not be disrupted, as shown by normal performance in a simple operant task, but that enhanced emotionality or fear of novelty as revealed in the open-field and tonic immobility tests may interfere with the expression of learning behaviour in some situations. However, changes in the general emotional state of an animal alone cannot easily explain why perceptual input for the 3h post-injection period is necessary for the cycloheximide or glutamate to alter subsequent behaviour on the pebble floor task (Rogers and Drennen, 1978; Sdraulig *et al.*, 1980).

Chickens injected with cycloheximide or glutamate were found to be hyperemotional (Part 3.2.1). We have previously shown that, in rats, emotionality correlates with the development of convulsive behaviour (Appendix 9, Ossenkopp and Sanberg, 1980). Furthermore, kainic acid injections into the striatum of rats have been shown to enhance seizure susceptibility (Appendix 10, Pisa *et al.*, 1980b). Therefore, it was of interest to determine

if chickens injected with cycloheximide or glutamate were more seizure-prone. The results reported in Part 3.2.2 demonstrate that, compared to controls these chickens are permanently more susceptible to the convulsive effects of pentylenetetrazol.

Enhanced susceptibility to seizures in chickens injected with cycloheximide or glutamate may directly underlie the learning deficits. Seizures which are provoked by specific or identified external stimuli are called reflex seizures (Forster, 1977). It is thought that reflex epilepsy reflects a learned triggering mechanism, resulting from a classically conditioned pairing of the seizure with an external stimulus (Burke, 1966). Indeed, paroxysmol spike activity has been conditioned to perceptual stimuli (Janowsky *et al.*, 1980). If paroxysmal spike activity were provoked by the pebble floor task, or even by the decision-making process itself, (Forster *et al.*, 1975) whether due to a conditioned pairing or not, this might be sufficient to disrupt normal performance.

Enhanced seizure activity as a direct result of the cycloheximide or glutamate injections may even be relevant to the genesis of the long-term lesions, and may help to explain the reported 'protection' effects of sensory deprivation (Rogers and Drennen, 1978; Sdraulig *et al.*, 1980), where perceptual input for the 3hr period after injection is necessary for the drugs to alter subsequent behaviour. It is possible that increased brain levels of the convulsant amino acids glutamate or aspartate, resulting from injections of cycloheximide, or glutamate itself, in neonatal chicks, may induce seizure activity, which in combination with more or less perceptual input and associated cerebral activity might cause greater or lesser

lesions, just as Ben-Ari *et al.* (1980) have found that kainic acid induces widespread cell lesions through the induction of epileptic activity, which can be prevented, at least in part, by the administration of diazepam to depress general neuronal activity. The reported protection effect of perceptual deprivation or anaesthetics (Drennen, 1977) in cycloheximide or glutamate-treated birds may simply be a result of decreased neuronal activity induced by a lack of perceptual arousal or increased sleep in the first case, and suppression of glutamate-induced seizuresin the latter case (cf. Zaczek *et al.*, 1978).

In rats, lesions of the striatum have been shown to alter various behaviours including arousal, memory, emotionality and seizure susceptibility (Sanberg *et al.*, 1978; Pisa *et al.*, 1980b; Appendix 10). Chickens injected with larger injections of cycloheximide or glutamate also show marked behavioural disturbances. It is possible that the paleostriatal pathology found in these birds (see Chapter 2) may underlie some of these behavioural deficits, particularly the enhanced emotionality and seizure susceptibility. However, in order to ascribe a role for the avian basal ganglia in psychological behaviour it is important that studies in which these nuclei are lesioned selectively be performed. Until such studies are completed, our interpretation can only be inferential.

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OPERANT, OPEN-FIELD AND TONIC IMMOBILITY BEHAVIOURS IN CHICKENS WITH FOREBRAIN INJECTIONS OF CYCLOHEXIMIDE OR GLUTAMATE

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to be published in

Behavioural Brain Research

SUMMARY

Chickens that had received bilateral injections of cycloheximide or glutamate into the forebrain on day 2 of life and tested 4 weeks later showed no deficit in acquisition, performance or extinction of continuously reinforced appetitive keypecking as compared to control birds injected with saline. However, chickens that had received injections of cycloheximide and were subsequently tested in an open-field apparatus took longer to leave the first square, defaecated more and pecked, preened and moved about less than controls. They also showed longer durations of tonic immobility. Those injected with glutamate exhibited similar behaviour but were not significantly different from controls in the open field latency to leave the first square, defaecation or tonic immobility tests. The above treatments have previously been described as producing permanent slowed learning in chickens on a pebble-floor task. Our results suggest that learning mechanisms may not be disrupted as shown by normal performance in a simple operant task but that enhanced emotionality or fear of novelty as revealed in the open field tests may interfere with the expression of learning behaviour in some situations.

Key words: Cycloheximide, glutamate, operant behaviour, open-field, tonic immobility, retarded learning, emotional reactions, fear.

INTRODUCTION

In 1972 Rogers and Mark (14) reported that intracranial injections of cycloheximide (a cytoplasmic ribosomal protein synthesis inhibitor), given during the first week after hatching, would subsequently render the animal incapable of learning at the normal rate on a pebbled floor task. Chicks injected with cycloheximide failed to reach the control levels of performance, that is they failed to choose predominantly grain, in preference to pebbles, in the latter half of the 60 pecks of the test. Sensory input (visual and auditory) within 3h following the injections were required to slow subsequent acquisition of these behavioural tasks. Cycloheximide-treated birds were also slower to habituate to visual and auditory stimuli. In addition, Rogers and Anson (12) have shown that birds treated with cycloheximide persisted in pecking at preferred food more than saline-injected control animals. Rogers and her colleagues (12, 15) have found the injection parameters of dose,volume and age of chicks to be critical in obtaining all these effects.

Hambley and Rogers (6) have postulated that cycloheximide produced these long term behavioural changes in chicks by the decreased utilization for protein synthesis and consequent accumulation of the amino acids, glutamate and aspartate. Forebrain injections of these neurotransmitters particularly glutamate, were shown to produce behavioural deficits on the pebble floor task qualitatively similar to those seen in the cycloheximide-injected birds (6, 23).

The factors that contribute to performance on the pebble floor are not clearly understood (10, 11). Therefore,

the present study was conducted in order to directly compare the long term effects of neonatal injections of glutamate and cycloheximide on a variety of behavioural tasks conventionally used to measure changes in learning (appetitive keypecking), activity (open-field) and emotionality (open-field and tonic immobility) in birds. A preliminary report has been presented elsewhere (22).

MATERIALS AND METHODS

A. Material

Birds and Housing.

White leghorn x Black Australorp male chickens were obtained from Research Poultry Farm (Victoria) on day 1 of life. They were housed in groups of 4-6 birds until day 14 when they were housed in pairs for the remainder of the study in 23 x 23 x 29cm metal cages with a clear perspex front. Warmth ($25^{o}C$) and light were provided continuously by overhead lamps (25W). Chicks had free access to grain (Hutmill, Victoria) and water (tap water with teramycin added) during the study, except when under deprivation schedules as described below.

Drug Administration.

On day 2 of life, the chicks were randomly assigned to three groups and given bilateral intracerebral injections of either cycloheximide (20µg/hemisphere), glutamate (210µg/hemisphere) or saline vehicle. The glutamate solution was buffered to pH 7.2 with Na_2PO_4. All injections were given in a vehicle of 25µl saline and performed freehand into the middle of each forebrain using a Hamilton 50µl syringe. A 26.5 gauge needle was used and fitted with a rubber stopper to prevent penetration beyond 3mm below the surface of the skull.

These doses and volumes were used since they were found to provide the optimal conditions for producing pebble floor deficits (12).

Appetitive keypecking.

Four standard BRS Foringer Skinner boxes for pigeons, enclosed in soundproof chambers, were used. Each was fitted with a centre illuminated pecking key located immediately above a grain feeder. They were modified with a grid floor of adjustable height, enlarged grain access hole and placement of a photocell beam across the food cubicle so that the beam was interrupted when the animal's head entered, as described elsewhere (16). Programming and stimulus delivery were controlled by Digital K-logic solid state circuitry. Key-pecks were recorded on printing counters converted from Royal 310P printing calculators. Photocell interruptions and reinforcements were recorded on counters converted from National Semiconductor 850A calculators (18). Chicken starter crumble was used as the reinforcer.

Open-field.

A 80 x 100cm platform with 50cm high walls was used as the open-field. It was painted white and the floor was divided by black lines into 20 squares of $20cm^2$. The open-field was placed in a 3.1 x 6.5m laboratory with fluorescent lighting 2.8m above the floor.

Tonic immobility.

Tonic immobility was measured on the floor of a BRS chamber with the door open for visual observation.

B. Methods

Acquisition and extinction of keypecking.

At three weeks of age, 16 cycloheximide-, 10 glutamate- and 14 saline-injected chickens were restricted to 1.5h of feeding daily sometime between 9:00pm and 11:00pm. One week later the animals were given a 20min magazine training session on both days 1 and 2. The animals were gently placed in the operant chamber, which had the grain feeder up and extra grain in the food hopper (days 1 and 2) and scattered on a paper towel on the grid floor immediately before the hopper entrance (day 1). On days 3-5, the animals were given hopper training over 20min sessions. On days 3 and 4 the feeder (hopper) delivered grain to the food cubicle for 10sec followed by 5sec of non-delivery. On day 5 food was delivered for 10sec, followed by 20sec of non-delivery. Photocell interruptions were measured over days 1-5. Days 6-10 consisted of autoshape-continuous reinforcement (CRF) training. The animals were given daily sessions of 30min each, in which the hopper delivered grain for 5sec every 30sec (days 6 and 7) or 60sec (days 8, 9 and 10). In each session the free grain delivery was preceded by 5sec of red illumination of the pecking key, which ceased when the grain was delivered (autoshape). In addition, the chicken received 5sec access to grain (CRF), if the key was pecked when not illuminated. A printing counter recorded those pecks which occurred during either the autoshape or CRF schedules over time (30sec periods for days 6 and 7; 60sec periods for days 8, 9 and 10). On days 11-19 the chickens were given CRF performance sessions only. These were 30min long; the pecking key was illuminated red continuously, and only grain (5sec access) contingent on keypecking was available. On days 20-24 reinforcement was discontinued.

Each of these five extinction sessions lasted until the chickens ceased to respond for three consecutive minutes. Both latency and number of responses to the extinction criterion were recorded.

Open-field.

At four weeks of age, 10 cycloheximide-, 10 glutamate- and 9 saline-injected chickens were placed individually in the central square of the open-field. For the next five minutes, the following behaviours were observed: 1) the latency (sec) to leave the first square; 2) the number of squares entered by both feet (ambulation); 3) the number of pecks at the floor; 4) the number of preening episodes; and 5) the number of times the animals defaecated. The open field was cleaned and wiped with a weak vinegar solution between each test.

Tonic immobility.

At four weeks of age, 19 cycloheximide-, 17 glutamate- and 20 saline-injected chickens were tested individually for tonic immobility. The bird was held down by hand on its right side for 15sec. Tonic immobility was measured as the time in sec from when the animal was released by the experimenter until it righted itself and rose to its feet. Additional induction attempts were given 60sec after each unsuccessful attempt to elicit tonic immobility. If the animal failed to show tonic immobility over five attempts it was given a score of zero sec. The floor was cleaned and wiped with a weak vinegar solution between each test.

Over all behavioural experiments, treatment and testing were completely randomized.

Statistics.

One way analyses of variance were used on the appetitive keypecking acquisition data, open-field and tonic immobility data. Bonferoni (7) multiple comparison tests were used to test significance between groups. Analysis of variance for trends of trial means (2) were performed on the appetitive keypecking acquisition and extinction data. Because of skewness and heterogeniety of variance on magazine and hopper training, and open field latencies, nonparametric Mann-Whitney U tests were used to test significance between groups. Two-tailed significance tests were used throughout the analyses.

RESULTS

Appetitive keypecking.

The median photocell counts for magazine and hopper training are shown in Figure 1. Only on day 1 was there any significant effect. The birds treated with cycloheximide broke the photocell beam in the food cubicle less often than birds treated with saline ($U = 61$, $n_1/n_2 = 14/16$, $p < 0.05$). The performance of birds treated with glutamate and those given cycloheximide ($U = 73$, $n_1/n_2 = 10/16$, $p > 0.05$) on this measure was not statistically different although visual observation suggested that the birds treated with cycloheximide ate much less grain on day 1 than either of the other groups.

The results of the autoshape-CRF are shown in Figures 2 and 3. The mean latencies in total min to the first keypeck (Figure 2) during the red illumination (autoshape) or non-illumination (CRF) periods were not significantly different between groups ($F = 1.59$, $df = 3.37$, $p > 0.05$, and $F < 1$, respectively). There were no differences between the mean

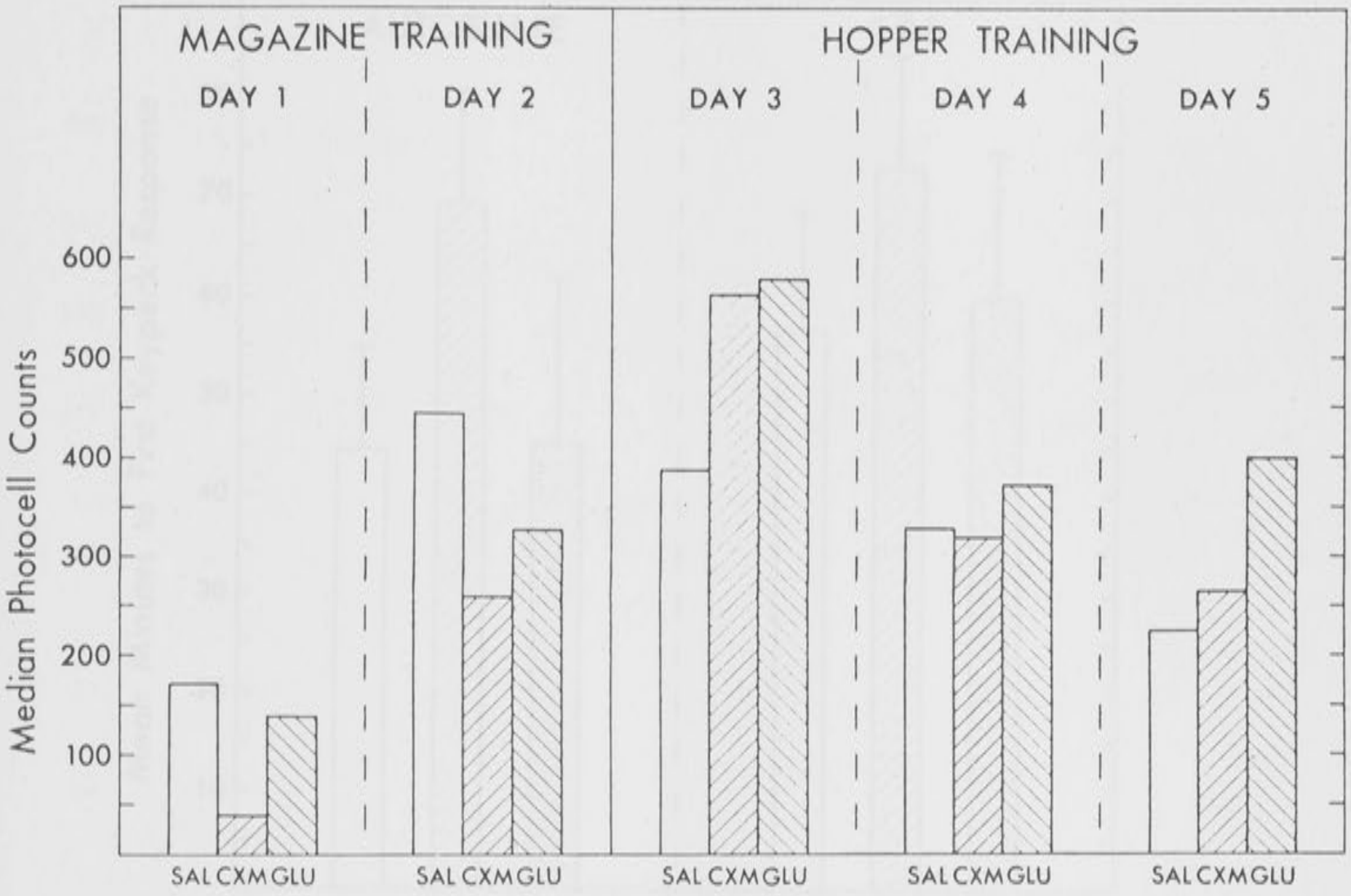

Figure 1. Median responses of head entries in food cubicle during acquisition of magazine and hopper training. SAL = saline group; CXM = cycloheximide group; GLU = glutamate group.

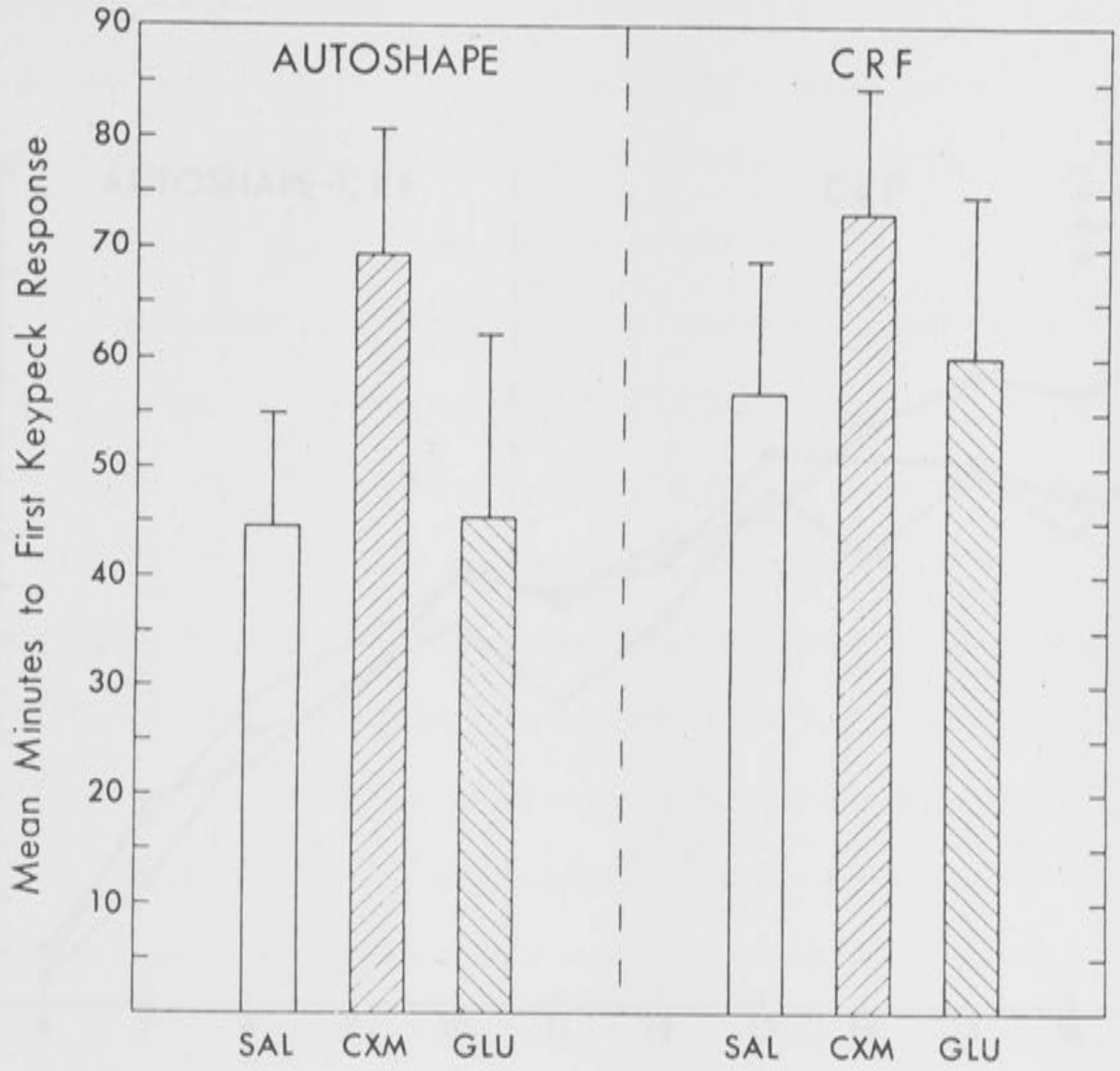

Figure 2. Mean time and standard error of the mean to first keypeck response in the autoshape or continuous reinforcement (CRF) periods during acquisition of continuously reinforced keypecking.

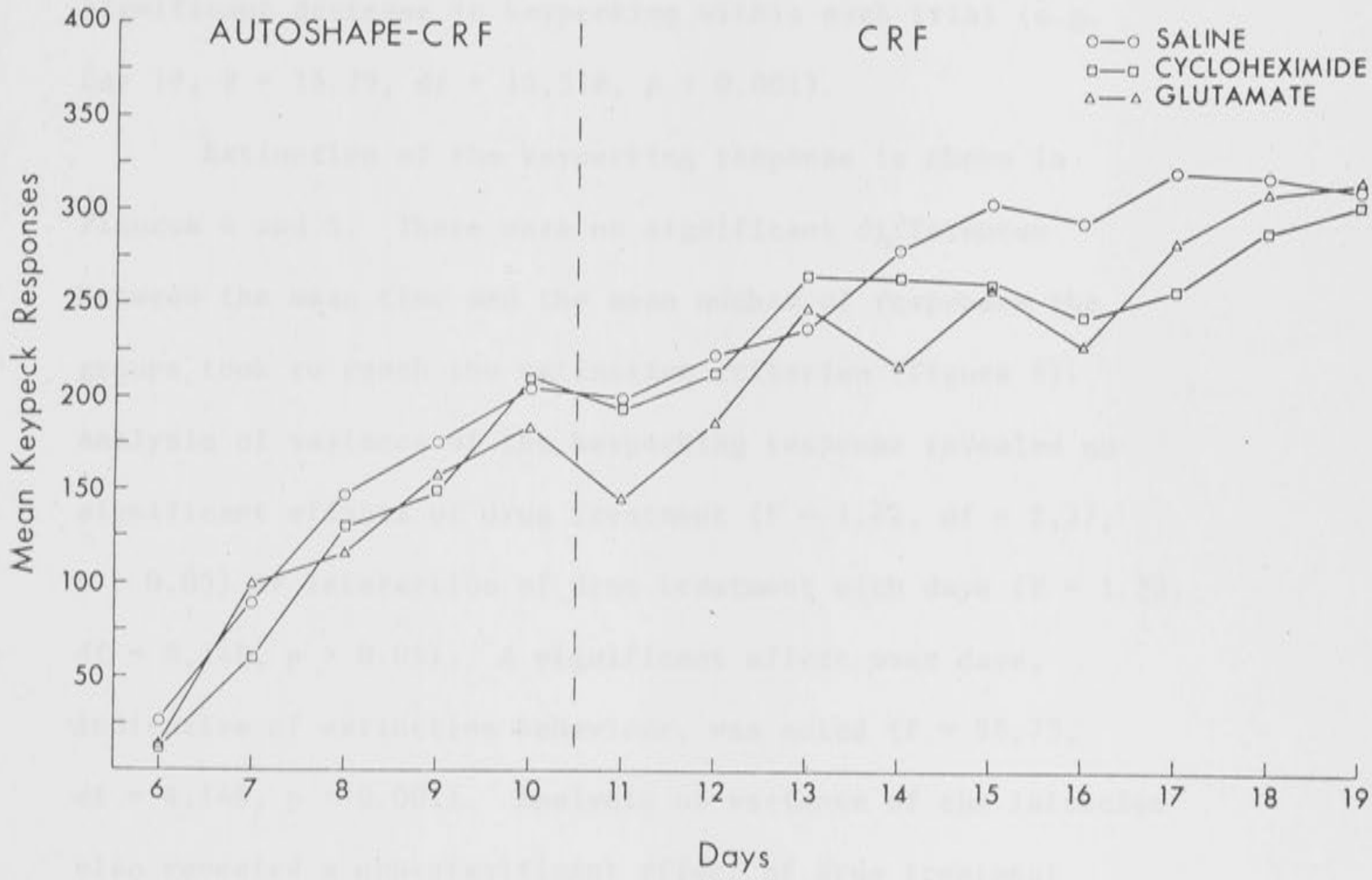

Figure 3. Mean responses during acquisition of continuously reinforced key pecking. Autoshape - CRF means the session days in which autoshaping procedures were used.

response rates of the different groups (Figure 3) ($F < 1$). All three groups showed a significant increase in the number of mean keypeck responses over the 13 days the birds were tested ($F = 52.4$, $df = 13, 481$, $p < 0.001$). On any one day the mean keypeck response did not differ significantly between the three groups (e.g. Day 19, $F < 1$). However, all three groups showed a significant decrease in keypecking within each trial (e.g. Day 19, $F = 15.79$, $df = 14,518$, $p < 0.001$).

Extinction of the keypecking response is shown in Figures 4 and 5. There were no significant differences between the mean time and the mean number of responses the groups took to reach the extinction criterion (Figure 4). Analysis of variance of the keypecking response revealed no significant effects of drug treatment ($F = 1.42$, $df = 2,37$, $p > 0.05$) or interaction of drug treatment with days ($F = 1.33$, $df = 8,148$, $p > 0.05$). A significant effect over days, indicative of extinction behaviour, was noted ($F = 98.73$, $df = 4,148$, $p < 0.001$). Analysis of variance of the latencies also revealed a non-significant effect of drug treatment ($F = 1.06$, $df = 2,37$ $p > 0.05$) and a significant effect over session days ($F = 241.75$, $df = 4, 148$, $p < 0.001$). There were no significant differences between groups on each extinction day over time (p's > 0.05). The results of extinction sessions on days 1 and 5 with time are shown in Figure 5.

Open-field.

Figure 6 shows that the birds treated with cycloheximide took significantly longer to leave the first square than birds treated with saline ($U = 17$, $n_1/n_2 = 10/9$, $p < 0.05$), however, birds given glutamate did not differ from cycloheximide- or

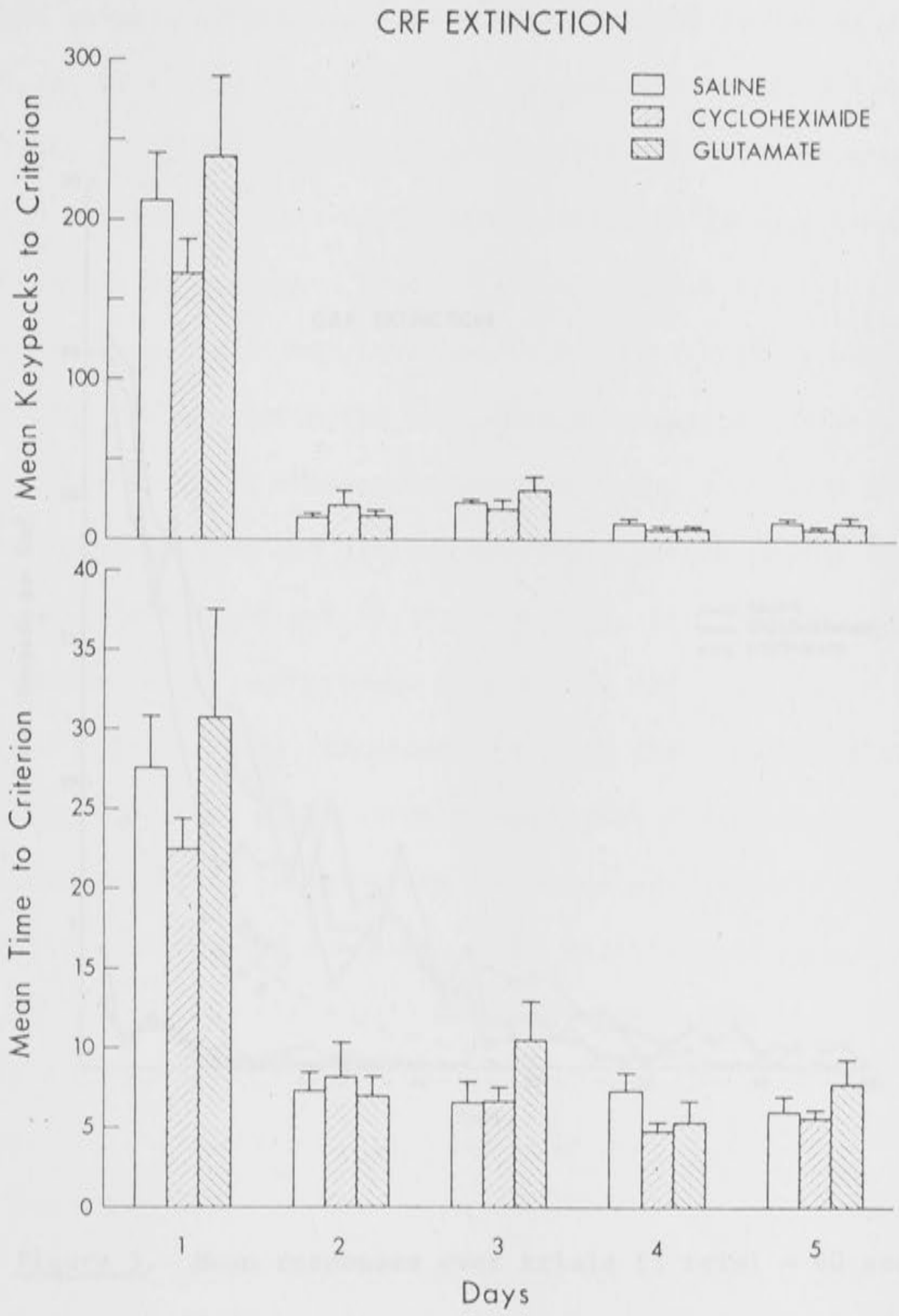

Figure 4. Mean responses and standard error of the mean (upper panel) and mean latency (sec) to criterion and standard error of the mean latency (lower panel) during extinction of continuously reinforced keypecking.

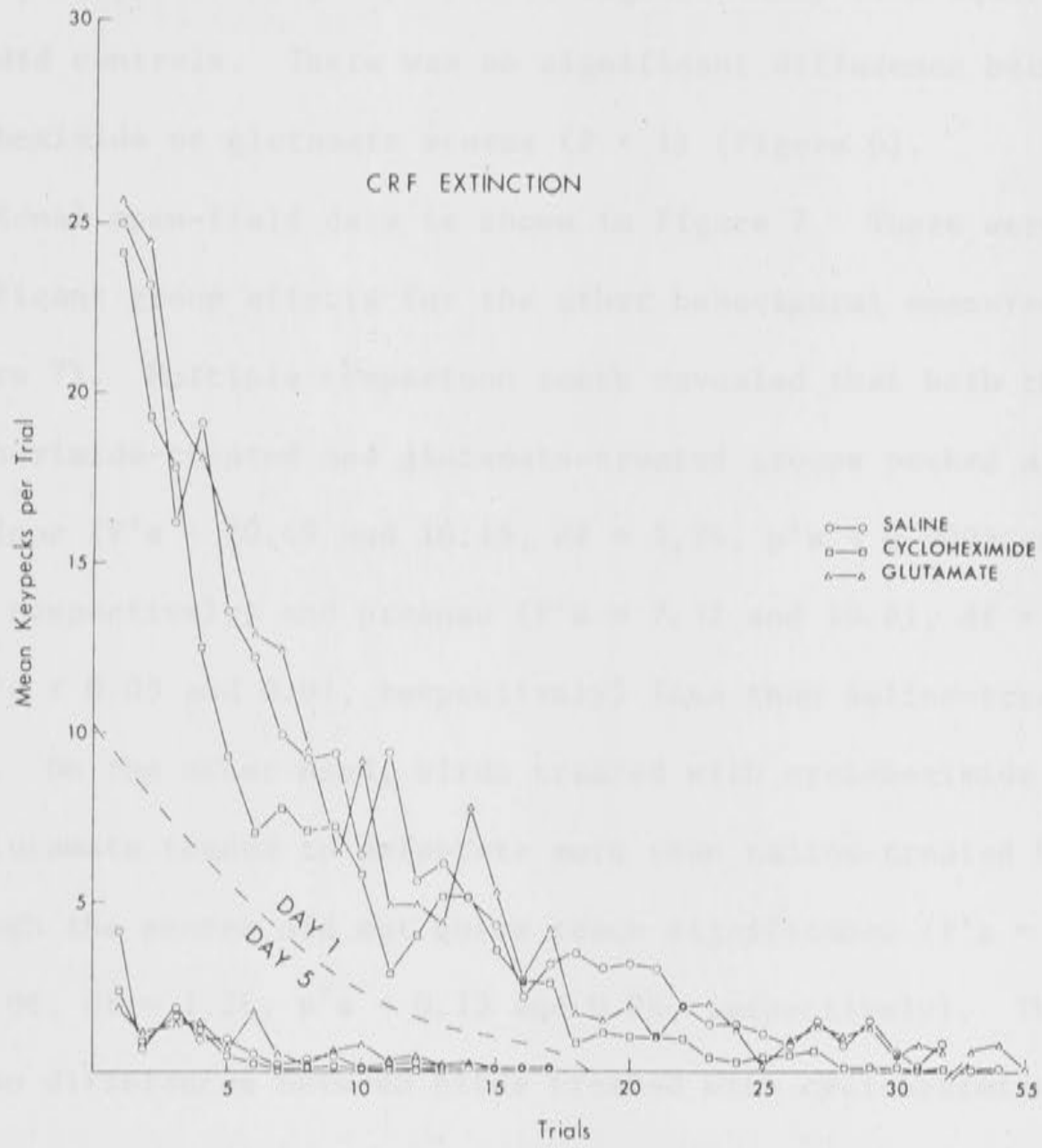

Figure 5. Mean responses over trials (1 trial = 60 sec) during extinction days 1 and 5 of continuously reinforced keypecking.

saline-treated animals (U = 48, n_1/n_2 = 10/10, and U = 28, n_1/n_2 = 10/9 respectively, p's > 0.05). There was a significant effect of groups on the ambulation measure (F = 5.3, df = 2, 26, p < 0.02). Multiple comparison tests revealed that both the cycloheximide- (F = 9.14, df = 1,26, p < 0.02) and glutamate-treated (F = 6.91, df = 1,26, p < 0.05) groups passed significantly less squares than did controls. There was no significant difference between cycloheximide or glutamate scores (F < 1) (Figure 6).

Additional open-field data is shown in Figure 7. There were significant group effects for the other behavioural measures (Figure 7). Multiple comparison tests revealed that both the cycloheximide-treated and glutamate-treated groups pecked at the floor (F's - 20.49 and 10.15, df = 1,26, p's < 0.0005 and 0.02, respectively) and preened (F's = 7.32 and 10.81, df = 1, 26, p's < 0.05 and 0.01, respectively) less than saline-treated birds. On the other hand, birds treated with cycloheximide and glutamate tended to defaecate more than saline-treated birds although the scores did not quite reach significance (F's = 4.48, and 6.06, df = 1.26, p's < 0.13 and 0.06, respectively). There were no differences between birds treated with cycloheximide and glutamate in these three behavioural measures.

Tonic immobility.

Analysis of variance of the tonic immobility results (Figure 8) showed a significant group effect (F = 3.42, df = 2,53, p < 0.05). Multiple comparison tests revealed a significant difference between cycloheximide- and saline-injected birds (F = 6.57, df = 1,53, p < 0.05). Birds injected with glutamate were not significantly different from either cycloheximide (F = 2.93, df = 1, 53, p > 0.05) or saline treated birds (F < 1).

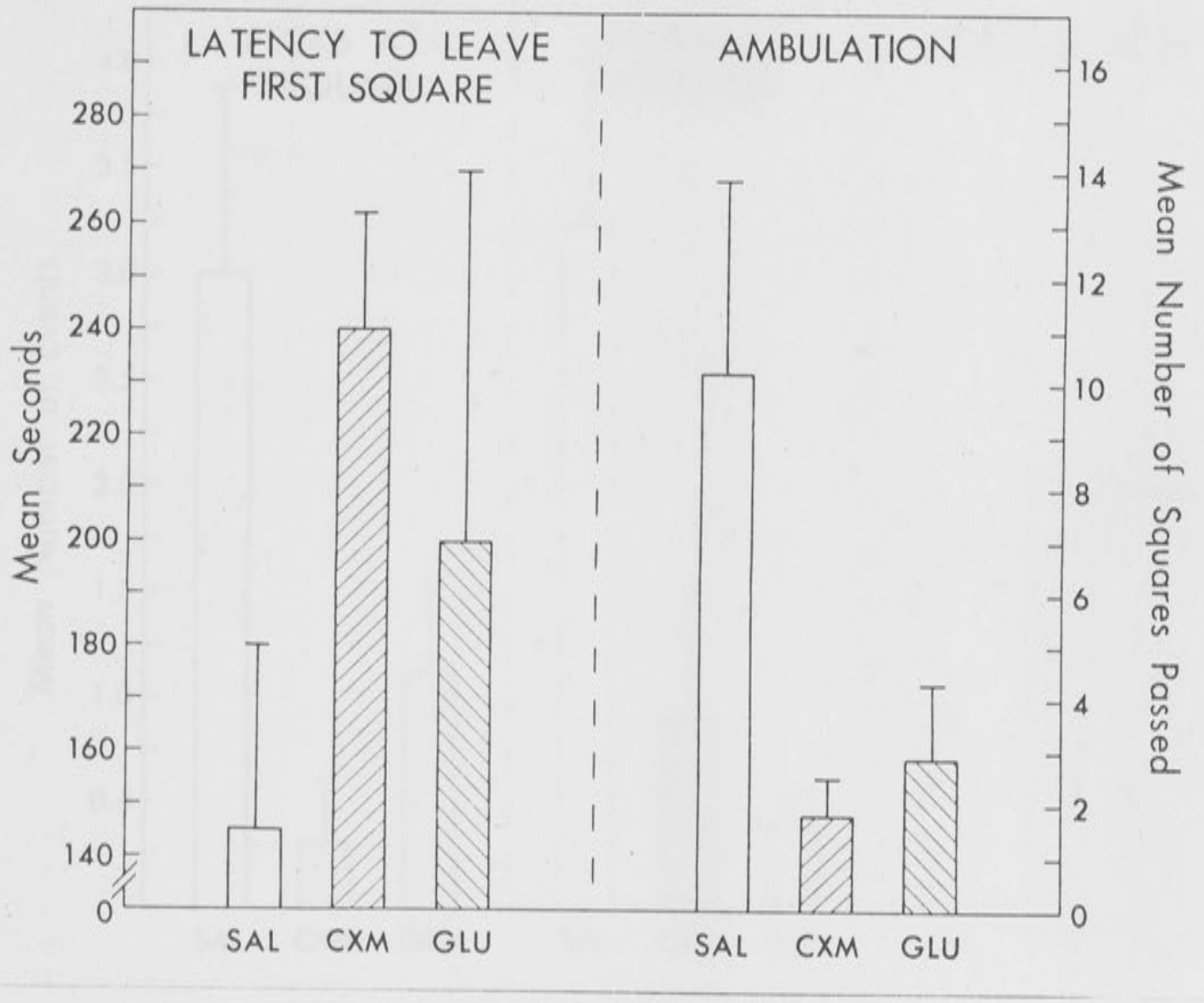

Figure 6. Latency to leave first square and ambulation in saline (SAL), cycloheximide (CXM) and glutamate (GLU) injected chicks tested for 5min in an open-field. Bars represent standard errors of the means.

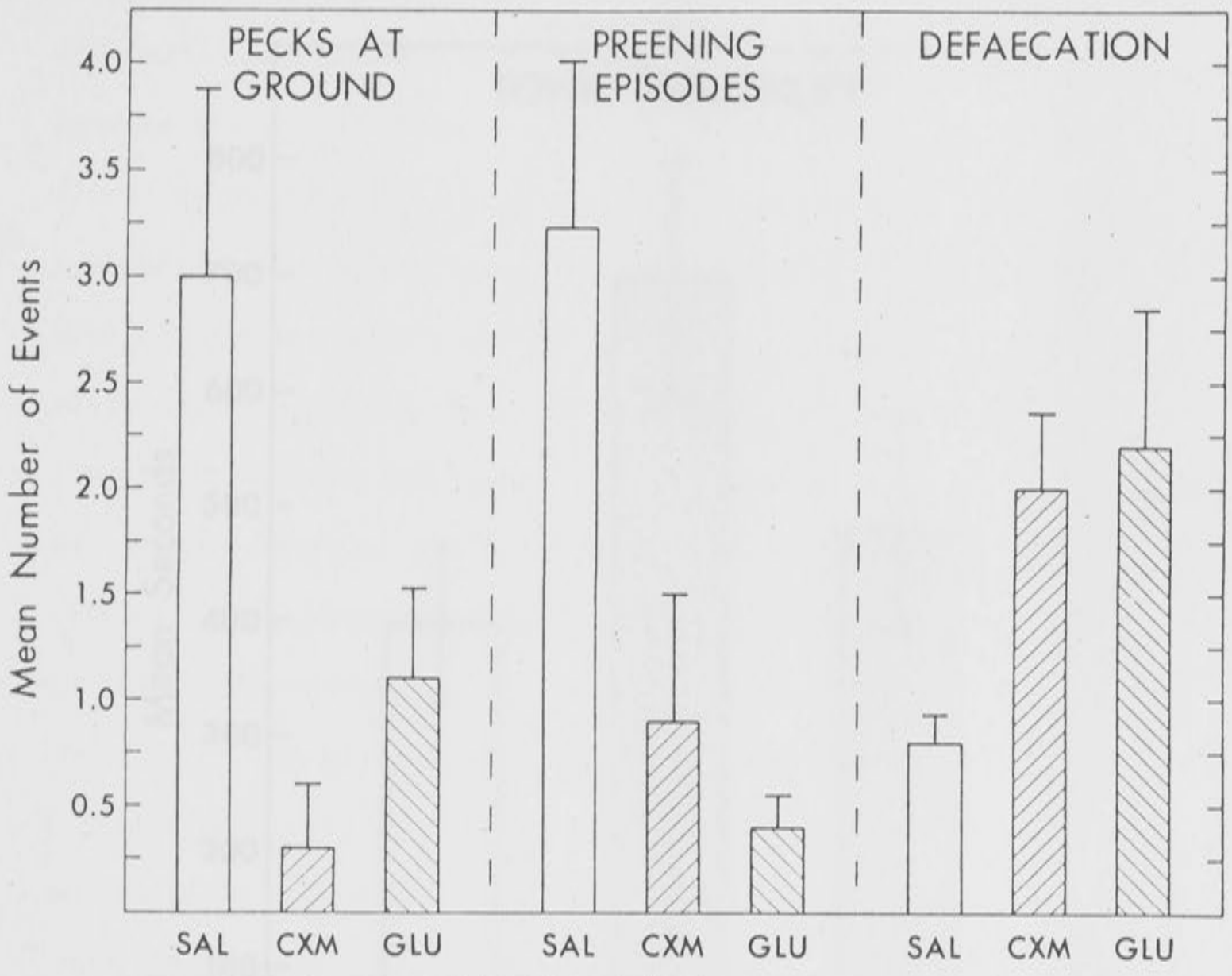

Figure 7. Number of pecks at ground, preening episodes and fecal boli in saline, cycloheximide and glutamate injected chicks tested for 5min in an open-field. Bars represent standard errors of the means.

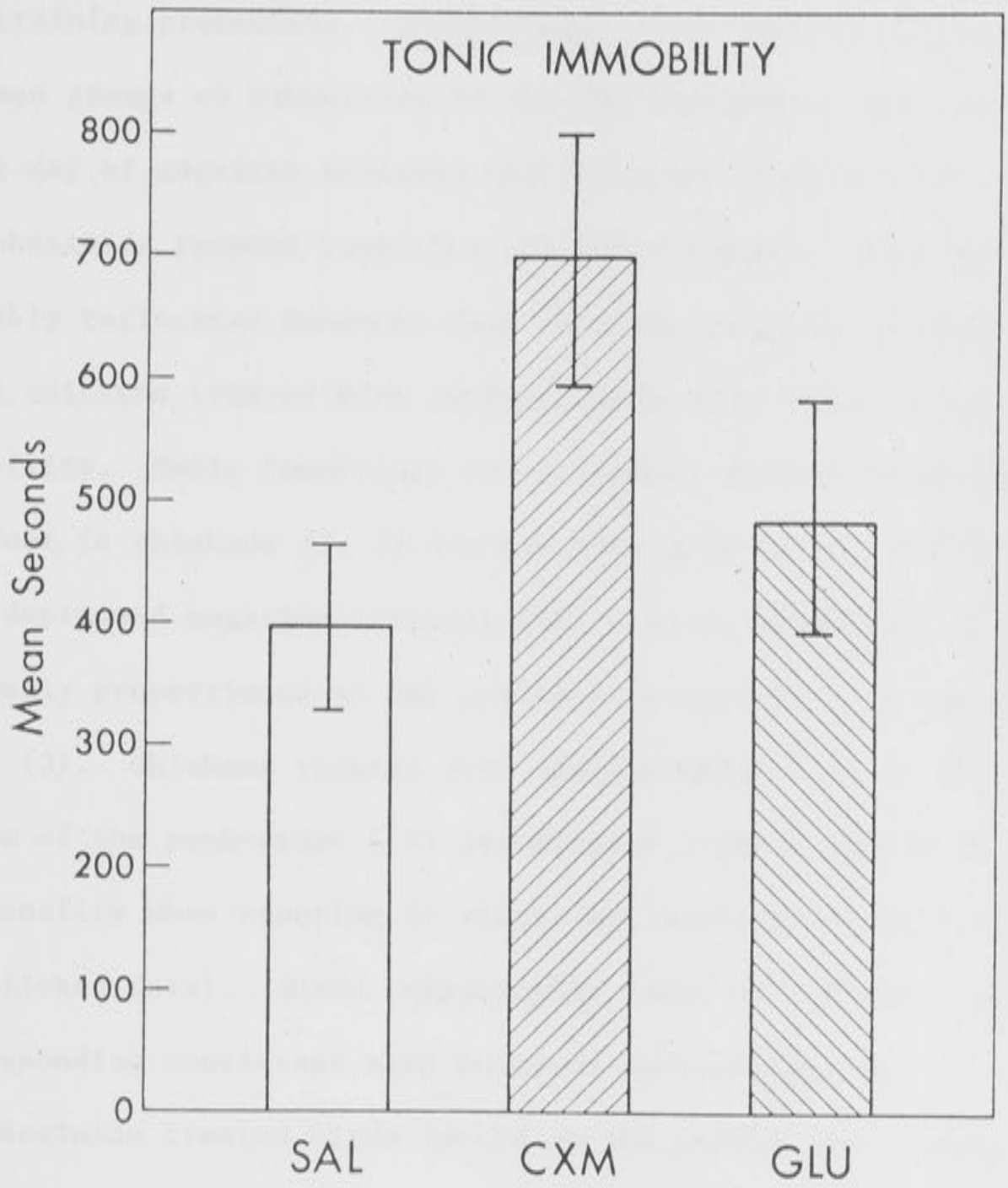

Figure 8. Duration of tonic immobility in saline, cycloheximide and glutamate injected chicks. Bars represent standard errors of the means.

DISCUSSION

Neonatal injections of cycloheximide or glutamate did not alter either rate of acquisition or asymptotic performance of keypecking behaviour in chickens. Regardless of treatment, all groups performed similarly throughout hopper, autoshaping and CRF training procedures. Furthermore, there were no differences between groups on extinction of the CRF schedule. Only on the first day of magazine training did the group treated with cycloheximide respond less than the other groups. This difference probably reflects an enhanced fear response to novel situations, since chickens treated with cycloheximide show enhanced tonic immobility. Tonic immobility is a reliable measure of emotionality and fear in chickens (3, 4) ans has been shown to be negatively correlated with depressed magazine training (19). Tonic immobility is also inversely proportional to the position of each bird in the peck-order (3). Chickens treated with cycloheximide fall to the bottom of the peck-order (15) and display altered levels of emotionality when reacting to visual and auditory stimuli (Anson, unpublished data). Anson (unpublished data) also found a pattern of responding consistent with enhanced emotionality in cycloheximide treated birds tested on the pebble floor task; that is, they took significantly longer to make the first five pecks than birds treated with saline.

Birds treated with cycloheximide or glutamate were less active in an open-field situation than controls as reflected by fewer ambulatory, pecking and preening episodes. This relative immobility may indicate increased fear and general emotionality, since both groups tended to defaecate more than saline-injected birds. Some investigators have questioned the use of defaecation as a measure of emotionality (1, 25), while

the latency to leave the first square is considered a better measure (9). Birds treated with cycloheximide, but not glutamate, showed significantly longer latencies to leave the first square than did controls. A similar pattern of results was also found in the tonic immobility experiment.

The differentiation between the cycloheximide and glutamate effects on the two emotionality measures of open-field latency and tonic immobility, compared to those induced by saline, could mean that the long term effects of these drugs are produced by different mechanisms. The fact that the differences between glutamate and cycloheximide over all behavioural tests were not significant may imply that the effects of glutamate injections alone are simply not as strong as cycloheximide-induced effects. Cycloheximide injections increase the brain levels of aspartate and other amino acids in addition to that of glutamate (6).

Previous studies with the pebble floor task (6, 12, 13, 14 15, 23) have shown that birds injected with cycloheximide or glutamate require many more pecks than saline-injected chicks to reach a similar level of performance. These results were interpreted as indicating that cycloheximide and glutamate interfered with associative and/or memory processes to produce slowed learning. In the present study, treatment with cycloheximide or glutamate had no significant effect on the rate of learning of an appetitive instrumental response. Perhaps alterations in pebble floor performance after treatment with cycloheximide or glutamate reflect not a learning deficit but altered emotionality. The results from the open-field study and the tonic immobility experiment suggest this could be the case. However, changes in the general emotional state of an animal alone cannot easily explain why (13, 23) perceptual

input for the 3h post-injection period is necessary for the drugs to alter subsequent behaviour on the pebble floor learning task. More work is needed before firm conclusions can be made about what effects cycloheximide and glutamate are having on the brains and behaviour of young chickens.

Originally, Rogers *et al.*, (15) reported that cycloheximide injections did not produce light or electron microscopic evidence of cellular damage in the hippocampal and Wulst areas. However, since glutamate is a known neurotoxic agent, especially in neonatal animals (5, 8) more detailed studies in other areas need to be carried out to elucidate the potential histological effects of cycloheximide and glutamate forebrain injections in young chickens. Previous research has shown permanent changes in neurotransmitter pharmacology following injection of kainate, an analogue of glutamate, into the rat striatum (17, 21) resulting in permanent changes in activity and emotionality (20). The known role of monoaminergic systems in emotionality (24) suggest that studies investigating permanent changes in this transmitter system following cycloheximide or glutamate injections may be fruitful.

ACKNOWLEDGEMENTS

The authors appreciate the excellent assistance of Drs. K. Gillette, J. Irwin, W.P. Bellingham, I.G. Morgan and K.E. Sanberg throughout the course of this study. This study was completed in partial fulfilment of the Doctor of Philosophy degree from the Australian National University to P.R. Sanberg.

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METRAZOL-INDUCED CONVULSIVE THRESHOLDS ARE REDUCED IN CHICKENS AFTER NEONATAL INJECTIONS OF CYCLOHEXIMIDE OR GLUTAMATE

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Submitted for publication to:

Experimental Neurology

ABSTRACT

Chickens which received forebrain injections of cycloheximide or glutamate when newly hatched were found to be more susceptible to metrazol-induced convulsions. Although these treatments do not produce overt convulsions by themselves some of the behavioural disorders that treated birds do show could be the result of sub-clinical paroxysmal activity.

Key words: Cycloheximide. Glutamate. Metrazol. Convulsions. Chickens.

An injection of cycloheximide (a cytoplasmic ribosomal protein synthesis inhibitor) into the forebrain of newly-hatched chicks has been shown to cause permanent slowing of learning in later life (13). It has been suggested that cycloheximide produced this effect in chicks as a result of an increase in brain glutamate and aspartate levels due to the decreased utilization of these amino acids for protein synthesis (7). Forebrain injections of these excitatory amino acid neurotransmitters have also been shown to subsequently render the chick incapable of learning at the normal rate on a pebbled floor task (7, 16). We have recently shown that these birds show heightened and disordered emotional responses, which could underlie the altered performances on the pebbled floor task (15). The present study was carried out to see if there were any alterations in seizure thresholds in the birds injected with glutamate or cycloheximide, since development of seizure behaviour has been found to correlate with emotionality in rats (10).

Two day-old White Leghorn x Black Australorp male chicks (Research Poultry Farm, Victoria, Australia) were randomly assigned to three groups and given bilateral intracerebral injections of either cycloheximide (20µg/hemisphere), glutamate (210µg/hemisphere) or saline vehicle. The glutamate solution was buffered to pH 7.2 with Na_2HPO_4. All injections were given in a volume of 25µl and were made freehand into the middle of each forebrain using a 50µl Hamilton syringe. A 26.5 gauge needle fitted with a rubber stopper to prevent penetration beyond 3mm below the surface of the skull was used. These doses and volumes were used since they were found to provide the optimum conditions for producing deficits in performance on the pebble

floor (7, 12). Birds were then housed in randomly mixed groups, with free access to grain (Hutmill, Victoria) and water (tap water with terramycin added). When 6 - 8 weeks old, they were injected intraperitoneally with either 50, 100 or 200mg/kg of pentylenetetrazol (Metrazol) in a volume of 1ml/kg distilled water. They were then placed individually in 22 x 24 x 28cm aluminium observation boxes with clear perspex fronts and tops. The convulsive behaviour of the birds was then measured for one hour.

Table 1 shows the results of convulsions produced by 50, 100 and 200mg/kg of Metrazol. At 50mg/kg of Metrazol, all the saline-treated birds were immobile, either standing or sitting, with their eyes usually closed. A significant number of cycloheximide-treated ($z = 2.07$, $p < 0.05$), and one of the glutamate-treated birds also showed myoclonic jerks of the body. One of the cycloheximide-treated birds exhibited a clonic convulsion involving wing-spreading and flapping. 100mg/kg of Metrazol induced significantly more cycloheximide-treated animals to have ictal responses (either a myoclonic jerk, or more rarely, a clonic convulsion) than controls ($z = 2.88$, $p < 0.01$). Typically, the myoclonic jerks merged into tonic-clonic convulsions of the limbs. Status epilepticus usually ensued leading to death in about 15min. The cycloheximide-treated group also had a greater proportion showing generalised convulsions ($z = 2.88$, $p < 0.01$) and death ($z = 3.33$, $p < 0.002$), than saline controls at 100mg/kg. The glutamate-treated birds tended to show a greater proportion of ictal responses and generalised convulsions than controls, but only the proportion of death was significant ($z = 2.00$, $p = 0.05$). At 200mg/kg there were no differences between groups in the proportions showing ictal responses, generalised convulsions or death. However,

TABLE 1: Metrazol-induced convulsions in saline, cycloheximide and glutamate injected chickens[†]

Dose	Saline	Cycloheximide	Glutamate
50mg/kg (n)	(5)	(5)	(5)
Proportion showing ictal responses	0%	60%*	20%*
100mg/kg (n)	(10)	(11)	(10)
Proportion showing ictal responses	30%	91%*	60%
Proportion showing generalised convulsions	30%	91%*	40%
Proportion which died	10%	82%*	50%*
200mg/kg (n)	(5)	(5)	(6)
Proportion showing ictal responses	80%	100%	100%
Latency to first ictal responses (sec)**	165 + 29.9	51 + 14.5*	66 + 29.3*
Proportion showing generalised convulsions	60%	100%	100%
Proportion which died	60%	100%	100%

*Significantly different from saline group, $p < 0.05$

**Data represent means $\pm$ standard error of the means

[†]The proportion data was analysed by Ferguson's test of the difference between two independent proportions (4). The latency data was analysed using one-way analysis of variance and Bonferoni multiple comparison tests (9).

the latency to the first ictal response was significantly different between groups ($F = 4.93$, $df = 2,12$, $p < 0.05$). Multiple comparison tests revealed that both the groups injected with cycloheximide and glutamate showed significantly shorter latencies to the first ictal response than the saline-treated birds ($Fs = 8.70$ and 6.52, respectively, $df = 1, 12$, $ps < 0.05$).

It is evident from the results that compared to controls, chickens injected with glutamate or cycloheximide on day 2 of life are permanently more susceptible to the convulsive effects of Metrazol. This is consistent with previous studies showing enhanced convulsive behaviour in more emotional animals (Ossenkopp and Sanberg, 1971), since chicks injected with cycloheximide and glutamate have also been shown to be hyperemotional (15). Birds injected with cycloheximide or glutamate show altered motor behaviours analogous to those in the rats (14), perhaps due to lesions of the avian homologue of the basal ganglia (the paleostriatum). In rats, lesions of the basal ganglia have been shown to increase seizure susceptability (11) and motor defects. It is possible that paleostriatal pathology in chickens injected with cycloheximide or glutamate may also underlie the enhanced seizure susceptability presently found in these animals.

Enhanced susceptibility to seizures may also underlie the learning deficits directly. Seizures which are provoked by specific or identified external stimuli are called reflex seizures (5). It is thought that reflex epilepsy reflects a learned triggering mechanism, resulting from a classically conditioned pairing of the seizure with an external stimulus (2). Indeed paroxysmal spike activity has been conditioned to perceptual stimuli (8). If paroxysmal spike activity were

provoked by the pebbled floor task, or even by the decision making process itself (6), whether due to a conditioned pairing or not, this might be sufficient to disrupt normal performance.

Enhanced seizure activity as a direct result of the injections may even be relevant to the genesis of the long-term lesions, and may help to explain the reported 'protection' effect (3, 16), where perceptual input for the 3h post-injected period is necessary for the drugs to alter subsequent behaviour. It is possible that increased brain levels of the convulsant amino acids glutamate or aspartate resulting from injections of cycloheximide or glutamate itself in neonatal chicks, may induce seizure activity, which in combination with more or less perceptual input and associated cerebral activity might cause greater or lesser lesions, just as Ben-Ari *et al.*, (1) have found that kainic acid induces widespread cell lesions through the reaction of epileptic activity, which can be prevented, at least in part, by the administration of diazepam to depress general neuronal activity. The reported protection effect of perceptual deprivation or anaesthetics in cycloheximide or glutamate treated birds may simply be a result of decreased neuronal activity induced by a lack of perceptual arousal or increased sleep in the first case, and suppression of glutamate induced seizure activity in the latter case (cf. 17).

Appreciation is extended to Dr. I.G. Morgan and Dr. J.M. Anson for help during the course of this study.

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CHAPTER FOUR

THE STRIATUM AND BODY WEIGHT

Chapter 4.

The Striatum and Body Weight

This chapter consists of the following parts:

Part 4.1 Chapter overview.

Part 4.2.1 Body weight, feeding and drinking behaviors in rats with kainic acid-induced lesions of striatal neurons - with a note on body weight symptomatology in Huntington's disease.

Part 4.2.2 New lesioning technique demonstrate that caudate-putamen neurons are involved in feeding and drinking behaviours.

Part 4.2.3 Body weight and dietary factors in Huntington's disease patients compared with matched controls.

Part 4.2.4 The avian paleostriatum and body weight: preliminary findings.

Part 4.1 Chapter overview

It has been known for several years that bilateral electrolytic lesions of the rat's lateral hypothalamus produce deficits in feeding and drinking behaviours such that, unless the animals are given special care, they will die from starvation and dehydration (Anand and Brobeck, 1951; Teitelbaum and Epstein, 1962). This is known as the lateral hypothalamic syndrome (LHS). Recovery from aphagia and adipsia occurs but the rats continue to exhibit chronic debilities such as absence or decrements in drinking in response to various regulatory challenges (Epstein and Teitelbaum, 1964), and a lowered body weight set point (Powley and Keesey, 1970). Destruction of extrahypothalamic neural tissue can also produce LHS-like regulatory deficits. Recent evidence has shown that destruction of fibers, particularly the dopaminergic fibers which traverse the lateral hypothalamus, plays an important role in the LHS (Baez *et al.*, 1977; Fibiger *et al.*, 1973). When the dopaminergic nigrostriatal tract is selectively lesioned by 6-hydroxydopamine, regulatory and body weight deficits very similar to the LHS result (Baez *et al.*, 1977; Fibiger *et al.*, 1973). Electrolytic lesions of the striatum, which also destroy the nigrostriatal terminals, result in aphagia, adipsia and decreased body weight (Neill and Linn, 1975; Schiff and Carter, 1977). In view of these findings, it was of interest to see if selective lesions of neurons postsynaptic to the dopamine terminals in the striatum result in a syndrome similar to that seen after nigrostriatal lesions.

The first paper (Part 4.2.1, Sanberg and Fibiger, 1979) explored the types of body weight and regulatory deficits which result from selective lesions of the non-dopaminergic neurons of the striatum in rats by kainic acid. In the first experiment (reported in Master's thesis; Sanberg, 1978) rats showed temporary aphagia and adipsia and their mean body weight was reduced following intrastriatal injections of 3nmole of kainic acid. Pettibone *et al.* (1978) have also shown that striatal interneurons are involved in eating and drinking behaviours as well as the maintenance of normal body weight. The abstract and accompanying figure (Sanberg *et al.*, 1979 and Figure 11) of Part 4.2.2 showed that these effects were dose-dependent, with a higher dose of 6nmole of kainic acid producing much greater deficits. On the basis of the previous findings showing that 6-hydroxydopamine lesions produce an LHS-like syndrome, it is reasonable that kainic acid lesions of the neurons postsynaptic to the dopamine terminals in the striatum also produce LHS-like changes. However, the results of experiment 1 (Part 4.2.1) also showed that rats treated with kainic acid behaved in a different manner compared to those with lateral hypothalamic or nigrostriatal lesions. Rats with striatal lesions, when deprived of food, increased their consumption of water. These results are the opposite of those reported for rats with either lateral hypothalamic or nigrostriatal lesions, in both of which large reductions in water intake were found during food deprivation (i.e., nonprandial drinking) (Baez *et al.*, 1977; Teitelbaum and Epstein, 1962).

Experiment 2 further examined the effects of food and water deprivation on feeding and drinking behaviours in rats with KA-induced striatal lesions. Although experiment 1 showed that there were no differences in water or food intake under free feeding and

and drinking conditions, when the rats with striatal lesions were deprived of food for 24h, their water intake was substantially greater than that of control rats. Experiment 2 confirmed these findings and showed that 24h food or water deprivation also caused rats with striatal lesions to consume more food during the subsequent 24hrs. The water deprivation results given in experiment 2 are presented in Fig. 9 at the end of the paper. Also, because of the significance of the non-prandial results as discussed above, this study was repeated for a third time and the results are shown at the end of the paper (Fig. 10). Although normal food intake in rats with striatal lesions was unimpaired, experiment 3 demonstrated motor deficits insofar as their ability to handle food pellets was greatly impaired. Finally, experiment 4 demonstrated that d-amphetamine and fenfluramine-induced anorexia was significantly enhanced in animals with lesions. In comparison, decreased amphetamine-induced anorexia and increased fenfluramine-induced anorexia are found in rats with lateral hypothalamic or nigrostriatal lesions under similar conditions (Blundell and Leshem, 1974; Fibiger *et al.*, 1973).

The general discussion of Part 4.2.1 describes the similarities of body weight regulation in rats with kainic acid-induced striatal lesions to that seen in Huntington's disease patients (see Chapter 5). The paper in Part 4.2.3 (Sanberg *et al.*, 1981) reports the results of a longitudinal study of body weight and dietary factors in Huntington's disease patients in comparison with a group of matched control subjects. It was found that even when eating high energy diets, loss of body weight is characteristic of Huntington's

disease patients. Further analysis showed that although some Huntington's disease patients initially may gain weight rapidly after a period in hospital, they eventually lose weight to below their admission levels. This pattern was not seen in the control group. In view of the animal work in Part 4.2.1, it is possible that the body weight symptomatology in Huntington's disease may be related to striatal degeneration. Therefore, body weight changes may be an important measure for evaluating the effectiveness of various therapeutic agents, or for estimating the degree of striatal pathology in Huntington's disease patients.

Compared to the extensive investigations on the neural mechanisms of body weight regulation in mammals (see Part 4.2.1), practically nothing is known about these mechanisms in birds. Kuenzel and his colleagues have shown that in the sparrow there are two hypothalamic nuclei involved in body weight regulation. As in rats (Mufson, 1980) they demonstrated that electrolytic lesions of the ventromedial nucleus resulted in hyperphagia and obesity, whereas bilateral lesions in the lateral hypothalamus caused aphagia and body weight loss (Kuenzel, 1972; Kuenzel and Helms, 1967, 1970). In chickens, Lepkovsky and colleagues have also shown aphagia and body weight loss after lesions of the hypothalamus. However, their lesions were not localised to any specific hypothalamic nuclei, except the general "posterior" region (Feldman *et al.*, 1957; Lepkovsky and Yasuka, 1966). The rather sparse available evidence thus suggests that birds have neurological mechanisms

controlling body weight similar to those seen in mammals. Because of a possible role of the mammalian striatum in body weight regulation (Part 4.2.1), in Part 4.2.4 body weight data from various striatal manipulative studies in chickens was analysed to see if these areas play a role in avian body weight regulation.

In the first study, chickens which received forebrain injections of cycloheximide or glutamate showed decreased body weight 2 weeks after injection. By 6 weeks, however, this mean difference was not evident, although the cycloheximide-treated group showed significantly greater variance than the control group. The disruption in body weight gains may be due to neurochemical pathology thought to occur in the paleostriatum of the cycloheximide or glutamate-injected chickens (see Chapter 2). However, because of the relatively gross nature of these injections it is also possible that diencephalic damage may have contributed to the results. Olney and Price (1978) have demonstrated that a single systemic injection of glutamate into neonatal mice produces initial weight loss followed by obesity. These injections in mice result in various diencephalic lesions. The significant effect of variance found in older cycloheximide-treated birds was a result of animals being very heavy or very light for their ages. It is possible that such effects are due to different degrees of diencephalic damage.

In the second study, removal of neostriatal and hyperstriatal areas in the chick did not produce any deficits in body weight. These areas are thought to be homologous to the neocortex of mammals (Karten and Dubbeldam, 1973). In rats,

cortical lesions do not produce any deficits in long-term body weight (Sanberg, unpublished observations). Preliminary work with kainic acid lesions of the chicken paleostriatum (the homologue of the mammalian striatum), however, demonstrated a significantly long term decrease in body weight in chickens. Therefore, as is rats, the avian "basal ganglia" may have a role in body weight regulation.

At present, work is proceeding on the role of the chicken paleostriatum in body weight regulation, by defining the resulting lesions histologically and biochemically. Regulatory tests, as used in Part 4.2.1, are being performed to elucidate the extent of the behavioural alterations. These studies in progress will allow better homologous comparisons between the avian and mammalian basal ganglia.

Because of the important role of the nigrostriatal dopamine system in mammalian body weight regulation, it will be important to determine if the avian homologue of this pathway, the tegmentopaleostriatal tract, also plays a role in avian body weight regulation. Goodman and Azzaro (1978) lesioned electrically this paleostriatal afferent pathway in pigeons, but they did not mention if any consummatory or body weight changes ensued. At present no study using 6-hydroxydopamine lesions in the bird have been performed. The effect of injections of 6-hydroxydopamine either into the tegmentopaleostriatal tract or the paleostriatum on body weight and regulatory behaviours may provide important clues into the homologies of the avian and mammalian basal ganglia.

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EXPERIMENTAL NEUROLOGY 66, 444–466 (1979)

Body Weight, Feeding, and Drinking Behaviors in Rats with Kainic Acid-Induced Lesions of Striatal Neurons—With a Note on Body Weight Symptomatology in Huntington's Disease[1]

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Received October 4, 1978; revision received June 7, 1979

Three nanomoles of kainic acid (KA) was injected into the striatum of rats to produce selective lesions in striatal neurons. Postoperatively the rats with lesions showed temporary aphagia and adipsia, and their mean body weight was reduced compared to sham-operated controls. Although a significant difference in body weight was maintained throughout the experiments, no significant differences were observed in *ad libitum* water or food intake between the animals recovered from lesions and the control animals. When the rats with striatal lesions were deprived of food for 24 h their water intake was substantially greater than that of control rats. Twenty-four-hour food or water deprivation also caused rats with striatal lesions to consume more food during the subsequent 24 h. Motor deficits were found in rats with lesions, insofar as their ability to handle the food pellets was greatly impaired. Finally, *d*-amphetamine and fenfluramine-induced anorexia was significantly enhanced in animals with lesions. The similarities and dissimilarities in the feeding and drinking behaviors between the rats with neostriatal lesions and those with lateral hypothalamic and nigrostriatal lesions are discussed. Biochemical and histological examinations confirmed the specificity of the KA-induced lesions in destroying only neuronal cell bodies although sparing axons and fiber bundles transversing or terminating in the striatum. Combined with previous results

Abbreviations: KA—kainic acid, LH—lateral hypothalamus, NSB—nigrostriatal bundle, GABA—gamma-aminobutyric acid.

[1] Supported by the Medical Research Council of Canada. The authors appreciate the excellent assistance of Ms. S. Atmadja, Dr. D. C. S. Roberts, Dr. M. E. Corcoran, Dr. E. G. McGeer, Mrs. K. Sanberg, Dr. S. T. Mason, Dr. M. Pisa, Ms. L. McSweeny, and Ms. A. Larson during the course of this study. Dr. Sanberg is now at the Department of Behavioural Biology, Research School of Biological Sciences, The Australian National University, Canberra, Australia.

0014-4886/79/120444-23$02.00/0

showing biochemical, histological, and psychological similarities between rats with KA-induced striatal lesions and humans with Huntington's disease, the present results further strengthen the parallels between this animal model and the human state for which decreases in body weight and regulatory deficits are also symptomatic.

INTRODUCTION

It is well known that lesions of the lateral hypothalamus (LH) produce deficits in feeding and drinking behaviors such that unless the animals are given special care, they will die from starvation and dehydration (3, 31, 52, 64). However, destruction of extrahypothalamic neural tissue can also produce LH-like regulatory deficits. For example aphagia and adipsia can result after lesions to the basal ganglia (1, 8, 18, 20, 21, 43, 44, 59, 63) and frontal cortex (27). In fact, recent evidence has shown that destruction of fibers, particularly the dopaminergic fibers which transverse the LH, plays an important role in the LH syndrome (1, 8, 18). Of particular importance is the dopaminergic nigrostriatal bundle (NSB). When this tract is selectively lesioned by 6-hydroxydopamine, regulatory deficits very similar to the LH syndrome result (1, 8, 18). Electrolytic lesions of the striatum, which also destroy the NSB terminals, result in aphagia, adipsia, and decreased body weight (21, 44, 59, 63). When the striatal nondopaminergic neurons (postsynaptic to the NSB) are selectively damaged by kainic acid, LH-like feeding and drinking regulatory impairments also ensue (48, 55).

Kainic acid (KA), a glutamate analog, is a relatively new neurotoxin which has been shown to rather selectively destroy neuronal perikarya, while tending to leave axons and terminals intact (12, 38, 60). When small amounts of KA are injected into the striatum the resulting pathology is considered to be representative of an animal model for Huntington's disease (12, 38, 60). Comparable to the pathology seen in postmortem tissue from patients with Huntington's (2, 4, 12, 29, 54), after KA-induced lesions the striatum also shows loss of intrinsic neurons, glial proliferation, intact afferent terminals and fibers of passage, marked shrinkage, and ventricular dilation (12, 34, 38, 55, 60). Biochemical similarities were established by the fact that striatal choline acetyltransferase and glutamic acid decarboxylase [synthesizing enzymes for acetylcholine and gamma-aminobutyric acid (GABA), respectively] are severely depleted, whereas tyrosine hydroxylase (synthesizing enzyme for dopamine) is relatively unaffected in both man (2, 12) and laboratory animal (12, 19, 34, 38, 55, 60). Psychological deficits, such as impaired learning, memory, and emotional reactions, as well as similar behavioral responses to various pharmacological manipulations, were found both in patients with Huntington's and in the KA-induced animal analog (34, 35, 50, 55, 57). Body weight and regulatory deficits were also shown to be associated with striatal KA lesions (46, 53),

thus further strengthening the parallels between this animal model and the human state for which such deficits are also symptomatic (2, 4, 6, 17, 24, 26, 30, 49, 51). At present little is known about the nature of the feeding and drinking impairments which result from KA-induced neostriatal lesions. In the present experiments we examined the short- and long-term consequences of KA-induced lesions of striatal neurons on body weight and feeding and drinking regulatory behaviors in the rat.

EXPERIMENT 1

The present experiment was essentially a replication of previous findings in which aphagia, adipsia, and body weight reductions were shown to follow KA-induced striatal lesions (48, 55). Water and food intake after recovery were also examined. It has been well documented that rats recovered from LH or NSB lesions display a pattern of drinking behaviors labeled "prandial drinking" (1, 18, 31, 64). Such rats consume water primarily to wet their mouths while eating dry food; therefore, when food is removed water intake (nonprandial drinking) is markedly reduced, compared to control rats. A test of nonprandial drinking in rats with KA-induced striatal lesions was made to examine any similarities to the LH syndrome.

Methods

Animals. Twenty male Wistar rats (305 to 350 g) were housed separately and given *ad libitum* food (Purina rat chow) and water (tap water). During the course of all experiments the animals were kept under a 12-h light:12-h dark schedule.

Surgery. Ten rats ($\bar{x}$ weight = 325 g) received bilateral stereotaxic injections of KA into the striatum. The rats were anesthetized with sodium pentobarbital (50 mg/kg) and 3 nmol KA in a volume of 0.5 μl phosphate-buffered saline, *p*H 7.4, was injected into each striatum. The coordinates from the interaural line and incisor bar at −4.2 were AP = +9.6 mm; ML = ±2.8 mm; and DV = +4.5 mm (28). The KA was injected via a 34-gauge cannula at a rate of 0.5 μl/3 min. After injection the cannula was left in place for a further 5 min to allow diffusion of the drug solution. Ten control rats ($\bar{x}$ weight = 333 g) underwent similar operations but received microinjections of the vehicle alone.

Procedure. Postoperatively, all animals were housed separately and given free access to food and water, and their body weights recorded daily. All animals with lesions were tube-fed intragastrically with 10 ml Soyalac twice daily, until they regained weight to at least 300 g. Control rats were handled similarly.

When the animals with lesions were all free-feeding, gaining weight, and had surpassed their preoperative body weights, graduated (100 ml) volumetric water feeders (Richter tubes) replaced the water bottles on all animal cages and daily water intake was recorded for 1 week. After the measurement of daily water intake all animals were food deprived 24 h. Then, the amount of food consumed in 20 min was recorded. Water intake during the 24-h food deprivation period was also measured in the Richter tubes.

After completion of the behavioral testing, eight rats with KA lesions and eight control rats were killed by cervical fracture and the brains dissected into various regions. In four experimental and four control brains the dorsal and ventral striatum, accumbens, and cortical regions were dissected and the activities of the following enzymes were assayed on these samples: choline acetyltransferase by the method of McCaman and Dewhurst (36), glutamic acid decarboxylase by the method of Chalmers *et al.* (9), and tyrosine hydroxylase by the method of McGeer *et al.* (40). In the other four KA-treated and four control brains, hippocampal and cortical noradrenaline contents were assayed by the method of McGeer and McGeer (37) on each of the samples. The remaining two experimental and two control rats were given an overdose of sodium pentobarbital and perfused intracardially with physiological saline followed by 10% Formalin. Sections were cut from frozen brain tissue at 50 μm and every third section was stained with cresyl violet. Two-way analysis of variance with repeated measures on one factor and Student's t tests (67) were used to determine the significance of the data.

Results

Aphagia and Adipsia. During the first 24 h after surgery, all animals with lesions showed loss of eating and drinking behaviors. This aphagic and adipsic behavior was temporary, lasting 1 to 3 ($\bar{x}$ = 1.8) days. Also during the first 24-h period most treated animals rejected even palatable pellet mash with sucrose added. For the first few postoperative days they exhibited intense stereotyped behaviors. During this same period approximately 40% showed bleeding from the urinary tract and nasal and visual orifices, which ceased after 1 to 3 days. Force feeding by gastric intubation was required to keep many of the animals with lesions alive. By the fourth postoperative day all animals were free-feeding on *ad libitum* food and water.

Body Weights. As seen in Fig. 1A the animals with lesions showed a significant and long-lasting depression of body weight relative to controls (groups F ratio (1,18) = 16.87, $P < 0.001$). After surgery, the control animals showed a continuous daily body weight gain (days F ratio (9,

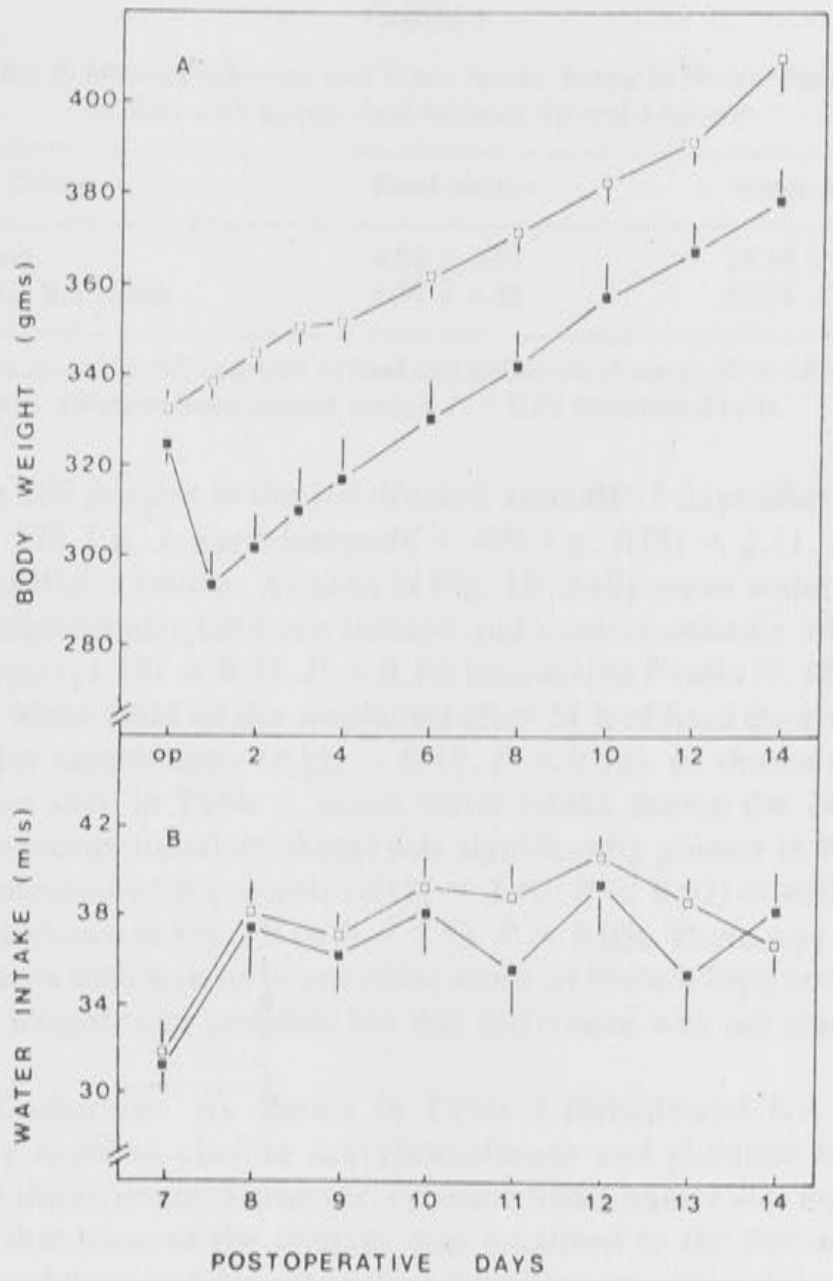

FIG. 1. Mean body weights (A) and mean water intake (B) for 10 rats with kainic acid-induced striatal lesions (solid squares) and 10 control (open squares) rats. Error bars represent 1 SE. Analysis of variance revealed significant differences in mean body weights between experimental and control rats for postoperative days. No significant differences were found for water intake between groups.

162) = 198.12, $P < 0.0001$). On the other hand, the lesion animals displayed an initial period of rapid weight loss (during the period of aphagia and adipsia) followed by daily weight gains at rates comparable to those of controls (interaction F ratio (9,162) = 8.84, $P < 0.001$). As seen in Fig. 1A, however, the animals with lesions never regained weight to control values, but instead, showed a relatively permanent reduction in body weight during the study. Although not shown in Fig. 1A, a significant reduction in body

TABLE 1

Food Intake for 20 Minutes following and Water Intake during 24 Hours' Food Deprivation in Rats with Kainic Acid-Induced Striatal Lesions[a]

Group	Food intake	Water intake
Control	4.63 ± 0.77	29.10 ± 2.62
Striatal KA lesion	4.77 ± 1.38	51.73 ± 7.71*

[a] Values are means ± SE in grams of food and milliliters of water. $N = 10$ in each group.
* Significantly different from control values, $P < 0.02$ (two-tailed test).

weight was still present in the KA-treated animals 75 days after surgery ($\bar{x}$ controls = 523.3 g, $\bar{x}$ experimentals = 499.3 g, $t(18) = 2.11$, $P < 0.05$).

Food and Water Intake. As seen in Fig. 1B, daily mean water intake did not differ significantly between treated and control animals after 1 week (groups F ratio $(1,18) = 0.55$, $P > 0.10$; interaction F ratio $(7, 126) = 1.04$, $P > 0.10$). Mean food intake measured after 24 h of food deprivation also did not differ significantly ($t(18) = 0.10$, $P > 0.10$), as shown in Table 1. However, as seen in Table 1, mean water intake during the 24 h of food deprivation (nonprandial drinking) was significantly greater in KA-treated animals compared with controls ($t(18) = 2.62$, $P < 0.02$) or with previous baseline day shown in Fig. 1B ($t(9) = 2.53$, $P < 0.05$). There was a tendency for the animals with lesions to consume more *ad libitum* food and water per gram body weight than controls but this difference was not significant on any day.

Lesion Evaluation. As shown in Table 2 intrastriatal KA injections significantly reduced choline acetyltransferase and glutamic acid decarboxylase in the striatum. However, tyrosine hydroxylase was not affected. It appears that most of the damage was localized to the dorsal striatum, with the transferase and decarboxylase activities approximately 60% of the control values in these regions. There was a significant reduction of the transferase activity in the ventral striatum whereas the decarboxylase activity was relatively normal. Except for a slight neuronal loss in cortical layers V and VI around the needle tract, all damage was localized in the dorsal striatum in the two brains observed. Gliosis, shrinkage, and intact fiber bundles were present in the striatal tissue. No damage could be seen in the lateral hypothalamus or substantia nigra. A further discussion can be found in experiment 4.

Discussion

Previous research has shown that lesions to the lateral hypothalamus in animals produce regulatory deficits, such as temporary aphagia and

TABLE 2

Choline Acetyltransferase (CAT), Glutamic Acid Decarboxylase (GAD), and Tyrosine Hydroxylase (TOH) Activities and Noradrenaline Concentrations in Control and Kainic Acid-treated Rats[a]

Area and group	CAT		GAD		TOH	
	Activity	Percentage[b]	Activity	Percentage[b]	Activity	Percentage[b]
Cortex						
Controls	15.2 ± 1.95		79.5 ± 2.18		0.12 ± 0.07	
Treated	15.4 ± 3.32	101	78.6 ± 3.46	99	0.13 ± 0.05	108
Accumbens						
Control	47.3 ± 6.57		136.8 ± 5.23		9.66 ± 0.99	
Treated	40.4 ± 2.85	86	147.4 ± 8.14	108	9.85 ± 0.44	102
Dorsal						
Control	72.8 ± 7.98		57.7 ± 4.47		9.47 ± 0.31	
Treated	43.3 ± 8.85*	60	37.7 ± 3.36**	65	10.56 ± 0.63	112
Ventral						
Control	66.6 ± 3.42		74.2 ± 4.09		9.50 ± 0.34	
Treated	44.7 ± 5.58**	67	68.9 ± 6.33	93	8.80 ± 0.28	93

	Hippocampal and cortical noradrenaline levels	
	Level	Percentage[b]
Control	0.37 ± 0.01	
Lesioned	0.34 ± 0.02	92

[a] The enzyme data are expressed as nanomoles per milligram protein per hour and noradrenaline values as grams per wet weight tissue. All values are means ± SE for four control and four experimental animals.

[b] Percentage of control activity remaining in kainic acid-treated tissue.

* Significantly different from control group, $P < 0.05$ (two-tailed Student's t test).

** Significantly different from control group, $P < 0.005$ (two-tailed Student's t test).

adipsia, and lowered body weights compared to sham-operated controls (3, 31, 52, 64). The severe change in body weight produced by LH lesions has been suggested to be due to a lesion-induced change in the body weight "set point" (52). It has also been shown, however, that selective lesions of the ascending dopaminergic fibers which transverse the LH produce regulatory deficits and decreased body weights similar to those found in animals with LH lesions (1, 18). The intrinsic neurons of the striatum receive their dopaminergic innervation from the substantia nigra via the NSB (41). On the basis of these previous findings it is reasonable that selective lesions of the neurons postsynaptic to the dopamine terminals in the striatum result in a syndrome similar to that seen after NSB lesions. Pettibone *et al.* (48), Sanberg *et al.* (55), and the present experiment showed that KA-induced lesions of these postsynaptic elements, which are characterized in part by cholinergic and GABAergic cells, are also involved in eating and drinking behaviors as well as the maintenance of

normal body weight. The long-lasting reduced body weight in KA-treated animals suggests that the loss of such striatal cells may cause a reduction in the body weight set point, as seen in rats with LH lesions. It is possible that the difference between choline acetyltransferase and glutamic acid decarboxylase activities in the ventral striatum is a result of cholinergic neurons being more sensitive than GABAergic neurons to the effects of KA. Alternatively, it is possible that there are cholinergic perikarya in the dorsal striatum which project to the ventral striatum. Histological examinations suggest that the latter alternative may be correct because cell loss was primarily restricted to the dorsal striatum.

The present results also show that the KA-treated rats had substantial differences compared to those observed in rats with LH or NSB lesions. In the present experiment it was demonstrated that rats with striatal lesions deprived of food increased their consumption of water. These results are opposite those reported for rats with either LH or NSB lesions, in both of which large reductions in water intake were found during food deprivation (i.e., nonprandial drinking) (1, 18, 31, 64).

EXPERIMENT 2

The previous experiment demonstrated that KA lesions of the striatum resulted in feeding, drinking, and body weight deficits which were in some respects similar to those seen after NSB and LH lesions. However, the increased water consumption in response to food deprivation observed in rats with KA-induced striatal lesions suggests marked differences also exist compared to rats with LH lesions, in which decreased drinking was found under similar conditions (1, 18, 31, 64). This second experiment further examined the effects of food and water deprivation on feeding and drinking behaviors in rats with KA-induced striatal lesions.

Methods

Animals. Eighteen male Wistar rats weighing approximately 300 g were used. They were maintained as decribed in experiment 1.

Surgery. Nine rats received bilateral injections of KA into the striatum exactly as described in experiment 1. Nine sham-operated rats served as controls.

Procedure. Postoperatively, the animals were handled as described in experiment 1 and recovered for 30 days prior to any behavioral testing. At the start of the behavioral studies the mean weights of the experimental and control groups were 390.6 and 409.5 g, respectively. In this experiment water intake was measured using standard water bottles and by calculating the difference in weights between designated periods. Food intake was

measured by calculating the difference between food weights for two periods, taking spillage through the bottom of the cages into account. The open spaces within the wire mesh were 1 cm^2.

Food Deprivation. Water intake was measured for 24 h, with food freely available (prandial drinking). The next day food was removed for 24 h and water intake during this period was measured (nonprandial drinking). At the end of the 24-h food deprivation period, two food intake tests and two water intake tests were given. These tests consisted of measuring food and water intake during 1 and 24 h after food deprivation under *ad libitum* conditions.

Water Deprivation. The procedures for this section are the opposite of those above. Food intake was measured for 24 h, with water freely available. Subsequently, water was removed for 24 h and food intake during this period was measured. After the water deprivation period two food intake tests and two water intake tests were conducted exactly as described in the previous section. Student's *t* tests (67) were used to determine the statistical significance of the data.

Results

Postoperative Observations. The rats with lesions were aphagic and adipsic after surgery for approximately 1 to 5 ($\bar{x}$ = 3.1) days. Three of the experimental animals showed temporary peripheral bleeding and all animals were tube-fed for at least 2 days or until they could maintain themselves on *ad libitum* food and water. Although not quantified in this study, abnormalities in grooming were evident in most rats as manifested by the marked yellowish discoloration and matting of the back fur.

Food Deprivation. Mean water intake during 24 h of available food (prandial) and 24 h of food deprivation (nonprandial) is shown in Fig. 2. The baseline intake in the presence of food was not significantly different between groups ($t(16) = 0.95$, $P > 0.10$). However, mean nonprandial water intake was significantly increased in the KA-treated group compared to controls ($t(16) = 3.08$, $P < 0.01$) and to their baseline day ($t(16) = 2.29$, $P < 0.05$).

The results of both the food and water tests are depicted in Fig. 3. Mean food intake for 1- in 24-h food-deprived rats did not differ between groups ($t(16) = 0.92$, $P > 0.10$). However, when food intake was measured for 24 h the KA-treated rats consumed significantly more food than did the controls ($t(16) = 2.17$, $P < 0.05$). Mean water intake measured for both 1 and 24 h, after 24 h of food deprivation, did not differ significantly between groups ($t(16) = 0.12$, $P > 0.10$ and $t(16) = 1.30$, $P > 0.10$, respectively).

Water Deprivation. Mean food intake for 24 h with or without available water did not differ between groups ($t(16) = 1.35$, $P > 0.10$ and $t(16)$

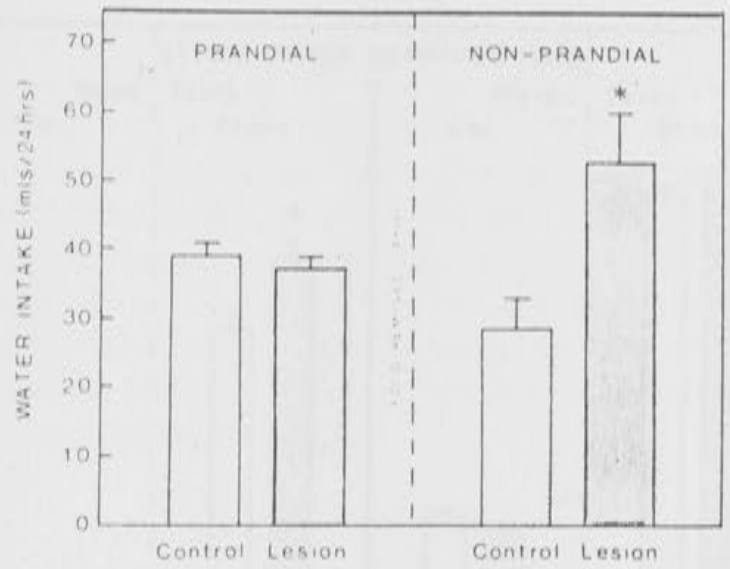

FIG. 2. Mean water intake (24 h) for nine control and nine rats with kainic acid-induced striatal lesions under conditions of available food (prandial) and no available food (non-prandial). Error bars represent 1 SE. $^*P < 0.01$ (two-tailed test).

= 0.56, $P > 0.10$, respectively). Both control and experimental animals showed decreased food consumption during water deprivation (control group $t(8) = 7.38$, $P < 0.0005$ and experimental group $t(16) = 6.41$, $P < 0.0005$).

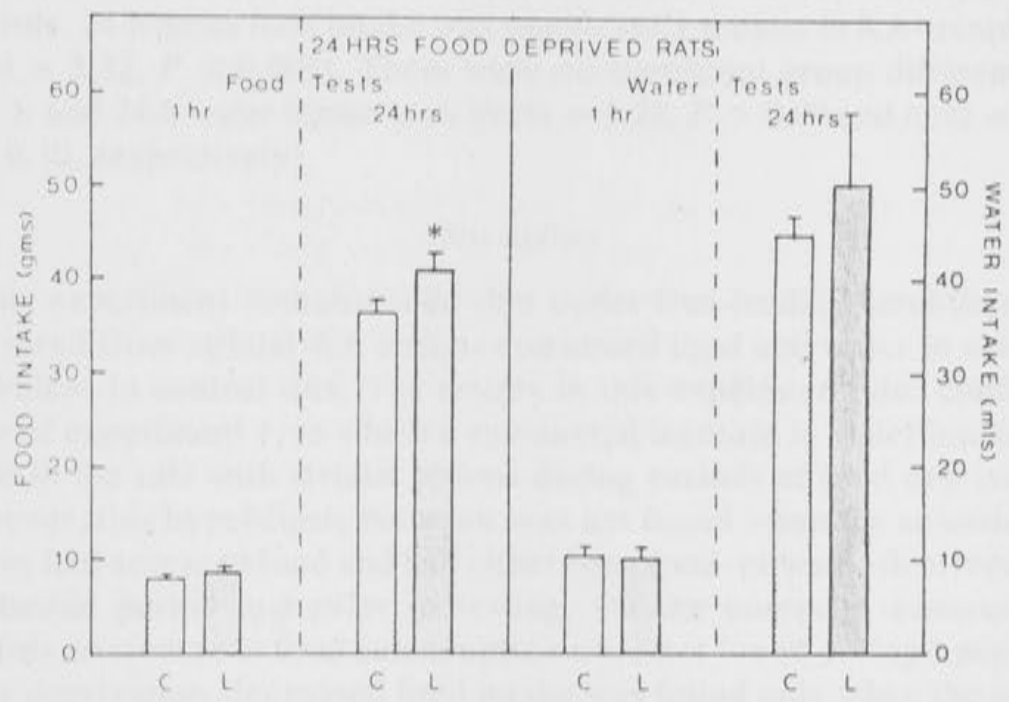

FIG. 3. Mean 1- and 24-h food and water intakes following prior 24-h food deprivation in control rats (C) and rats with kainic acid-induced striatal lesions (L). Error bars represent 1 SE. $^*P < 0.05$ (two-tailed test).

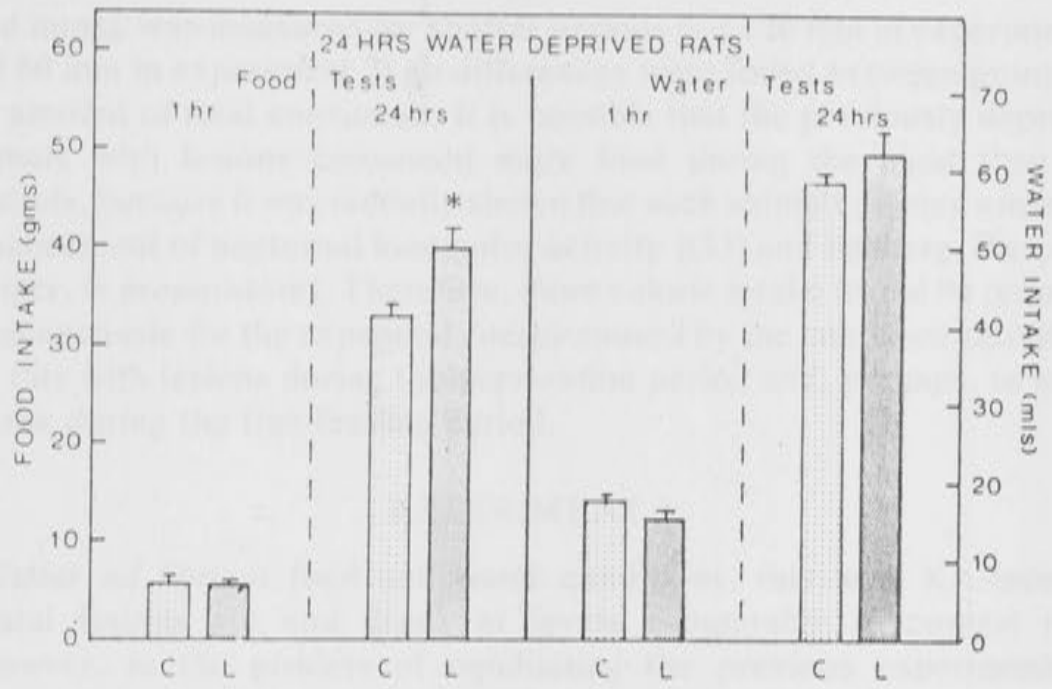

FIG. 4. Mean 1- and 24-h food and water intake following prior 24-h water deprivation in control rats (C) and rats with kainic acid-induced striatal lesions (L). Error bars represent 1 SE. $^*P < 0.005$ (two-tailed test).

Figure 4 shows the results of the food and water tests in 24-h water-deprived rats. Mean 1-h food intake was not different between KA-treated and control rats ($t(16) = 0.14$, $P > 0.10$). Compared to controls, 24-h mean food intake was significantly greater in KA-treated rats ($t(16) = 3.32$, $P < 0.005$). There were no significant group differences in both 1- and 24-h water intake tests ($t(16) = 1.72$, $P > 0.09$ and $t(16) = 1.25$, $P > 0.10$, respectively).

Discussion

This experiment demonstrated that under free-feeding conditions rats recovered from striatal KA lesions consumed food and water in amounts equivalent to control rats. The results in this experiment also confirmed those of experiment 1, in which a substantial increase in water intake was found in the rats with striatal lesions during periods of food deprivation. However, this hyperdipsic behavior was not found when the animals with lesions had access to food and had either been food- or water-deprived for a substantial period just prior to testing. On the contrary, compared to controls, increases in food consumption were not found during a period of water deprivation. Increased food intake was found only when the animal had been previously food- or water-deprived and had access to water during testing. This increased food consumption occurred only when the animal was allowed to eat for an extended period of time (i.e., 24 h). When

food intake was measured for shorter periods (i.e., 20 min in experiment 1 and 60 min in experiment 2) no differences were found between groups in the amount of food consumed. It is possible that the previously deprived animals with lesions consumed more food during the night than did controls, because it was recently shown that such animals display a marked enhancement of nocturnal locomotor activity [(33) and Sanberg, Pisa, and Fibiger, *in preparation*]. Therefore, more caloric intake would be required to compensate for the expended energy caused by the increased activity of the rats with lesions during the deprivation period and, perhaps, to some degree during the free-feeding period.

EXPERIMENT 3

Under *ad libitum* food and water conditions, rats with KA-induced striatal lesions ate and drank at levels comparable to control rats. However, in the process of conducting the previous experiments it appeared that experimental rats spilled more food through the bottom of the cages than did controls. Furthermore, the appearance and size of the remaining food pellets also differed between groups. This suggested that recovered free-feeding rats with striatal lesions may have an eating behavior different from that of control rats. The present experiment examined the differences in their pattern of food consumption.

Methods

Animals. Animals used in this experiment were those used in experiment 2 after they had been replaced on an *ad libitum* diet for 1 week following experiment 2.

Procedure. Ten food pellets (Purina rat chow) were placed in each animal's cage. The mean weight of the 10 food pellets was calculated and separate cardboard trays were placed under each cage to collect spillage. The animals ate the food pellets for 24 h (water available) and then the number and mean weight of the pellets remaining in each cage were determined. The food spillage was also weighed. Representative photographs of the remaining food were taken.

Results

Figure 5 shows the results of 24 h of free-feeding on 10 initial food pellets. Figure 5A shows that no differences were found in the mean number of food pellets remaining in the cage 24 h later ($t(16) = 0.54, P > 0.10$). Figure 5B shows that the mean weight of the remaining food pellets did, however, differ between groups. The pellets in the experimental rats' cages weighed significantly less than those remaining in control cages ($t(16) = 2.86$,

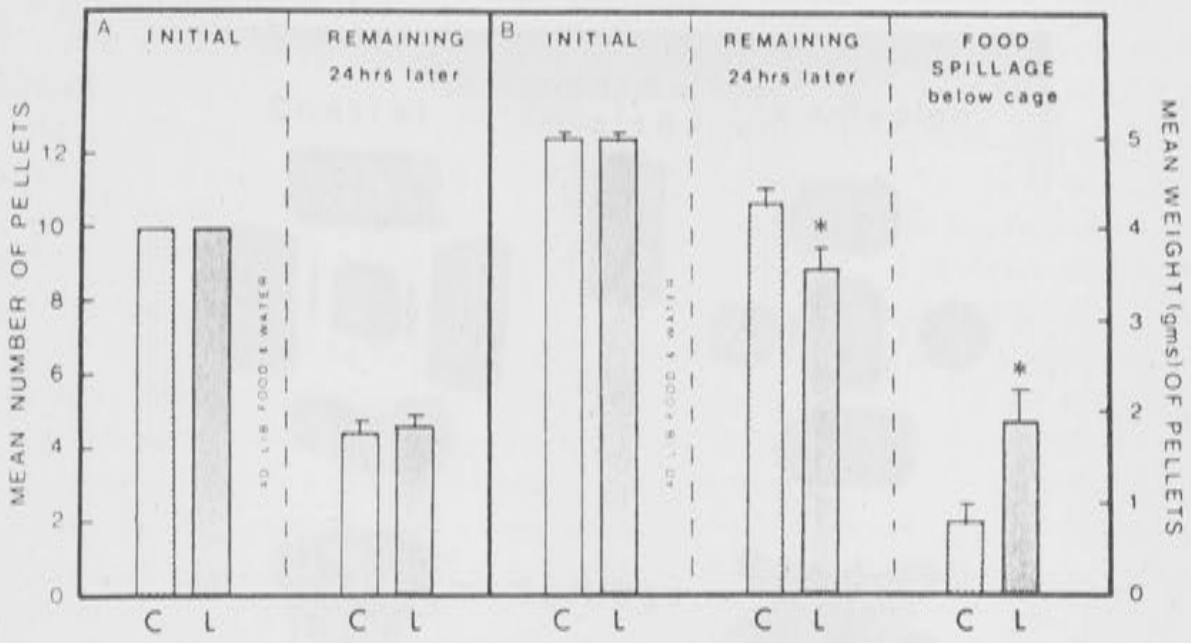

FIG. 5. Mean number (A) and mean weight (B) of food pellets (initial) after 24-h free-feeding (remaining) in nine control rats (C) and nine rats with kainic acid-induced striatal lesions (L). Error bars represent 1 SE. *$P < 0.02$ (two-tailed test).

$P < 0.02$). Conversely, more food spillage was found below the experimental cages than below the control cages ($t(16) = 2.67$, $P < 0.02$). Mean food intake did not differ between groups (experimental $\bar{x} = 34.8$ and control $\bar{x} = 32.1$; $t(16) = 1.36$, $P > 0.10$). Figure 6 gives a pictorial representation of the difference in appearance between the food pellets remaining in the control and experimental animals' cages as well as the spillage found at the end of the experiment. The remaining pellets were smaller and rounder for the experimental animals. Concurrently, larger pieces of food pellets were found in the spillage of animals with lesions, compared to the powdery spillage of control rats. Observation showed that rats with KA-induced striatal lesions had difficulty handling the food pellets between their forepaws when the corners were chewed off and so proceeded to initiate feeding on a fresh food pellet. As a result the remaining food pellets in the experimental animals' cages, when observed 24 h later, had mostly all been gnawed. The normal pattern of control rats was to eat one pellet completely and then proceed to another.

Discussion

The results of this experiment indicated that although *ad libitum* food intake did not differ significantly between control rats and rats with striatal lesions, their patterns of eating differed. The tendency of experimental rats to chew at more pellets than did the controls may have resulted from a motor deficit in their ability to handle small food pellets without distinctive

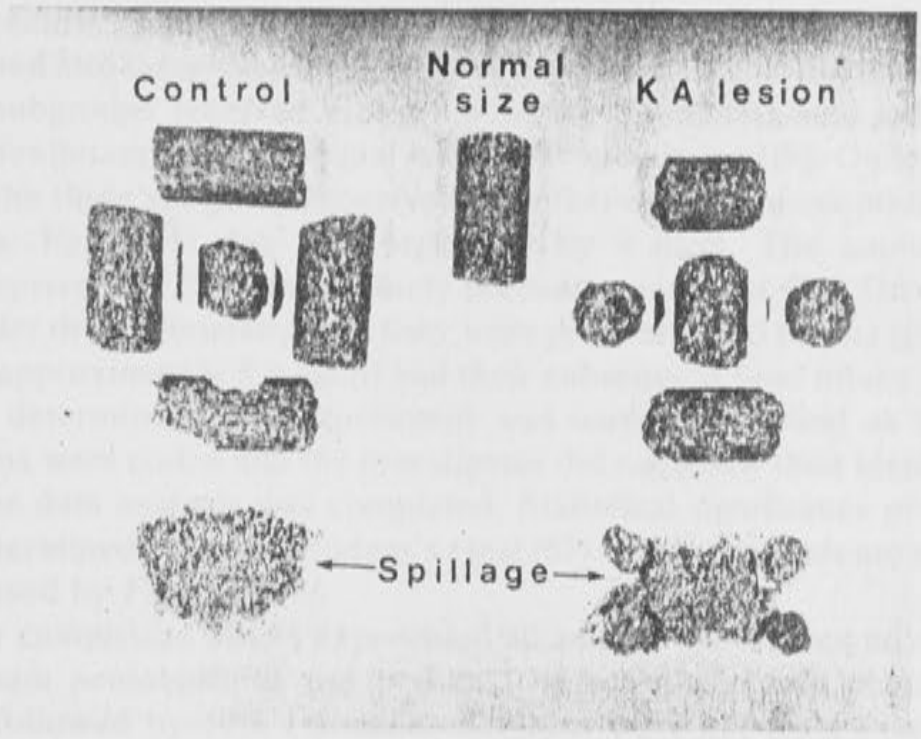

FIG. 6. Representative sample of initial food pellets (center) after 24-h free-feeding in control rats (left) and rats with kainic acid-induced striatal lesions (right). Spillage found below cages is at bottom of pictures.

corners to grab on to. Alternatively, the increased chewing may be a form of stereotyped gnawing behavior caused by increased dopaminergic activity which has been shown to occur in these animals. It is of interest that lesions of the frontal cortex, an area which projects to the striatum (22), also resulted in *short-term* eating and motor impairments similar to those found in the rats with KA-induced striatal lesions (27).

EXPERIMENT 4

In rats with NSB and LH lesions it was shown that anorexia induced by *d*-amphetamine sulfate is markedly reduced (3, 8). This is because the dopaminergic NSB is a major site of action of amphetamine (25). Conversely, the anorexic action of fenfluramine, a presumed serotonergic agonist (25), is relatively enhanced in animals with NSB or LH lesions (3, 18). The present experiment examined the anorexic effects of both *d*-amphetamine and fenfluramine on rats with KA-induced striatal lesions.

Methods

Animals. The rats used in the previous experiment were rested 5 days and then used as subjects for this experiment. Their mean weights were 436.8 g (controls) and 421.0 g (experimental) on test day 1.

Procedure. The experimental and control groups were randomly separated into three subgroups of three rats each. On test day 1 each of the three subgroups received either 1.5 mg/kg *d*-amphetamine sulfate, 3.0 mg/kg fenfluramine, or an equal volume of vehicle (water). On test days 2 and 3 the three subgroups received the other drugs in a counterbalanced fashion. Each test day was separated by 4 days. The animals were food-deprived for 24 h immediately preceding each test day. On each test day, after drug administration, they were given six food pellets (Purina rat chow, approximately 5 g each) and their subsequent food intake for a 2-h period determined. The experiment was carried out blind as the drug solutions were coded and the investigator did not know their identity until after the data analysis was completed. Statistical significance of the data was determined by use of Student's *t* test (67). These methods are similar to those used by Fibiger *et al.*

After completion of this experiment all animals were given an overdose of sodium pentobarbital and perfused intracardially with physiological saline followed by 10% Formalin. Sections were cut from frozen brain tissue at 60 μm and every third section was stained with cresyl violet.

Results

As depicted in Fig. 7, food intake for 2 h following 24 h of food deprivation did not differ under baseline (water) conditions ($t(16) = 0.77$, $P > 0.10$). However, pretreatment with 1.5 mg/kg *d*-amphetamine or 3.0 mg/kg fenfluramine produced significantly greater reduction in food intake

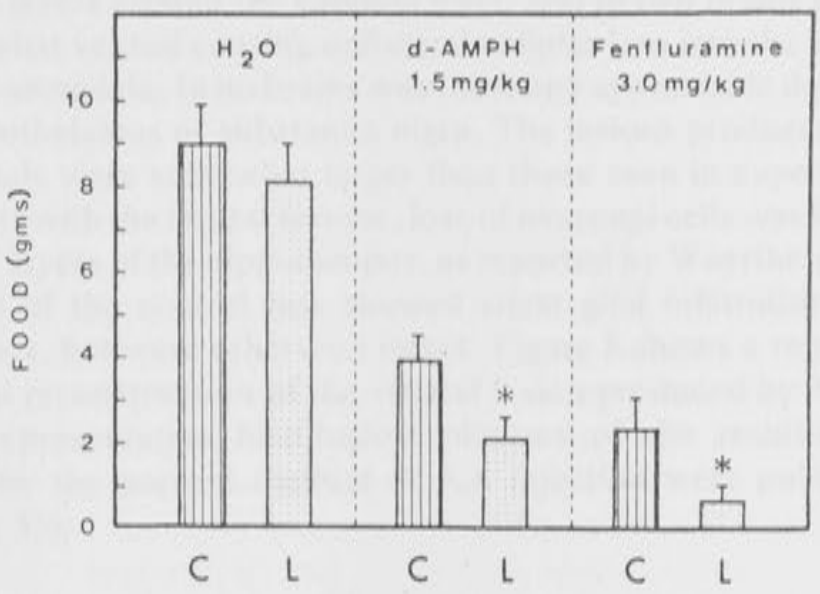

FIG. 7. Mean 2-h food intake after intraperitoneal injection of water, 1.5 mg/kg *d*-amphetamine sulfate, or 2.0 mg/kg fenfluramine in 24-h food-deprived control rats (C) and rats with kainic acid-induced striatal lesions (L). $N = 9$ per group. Error bars represent 1 SE. $^*P < 0.05$ (two-tailed test).

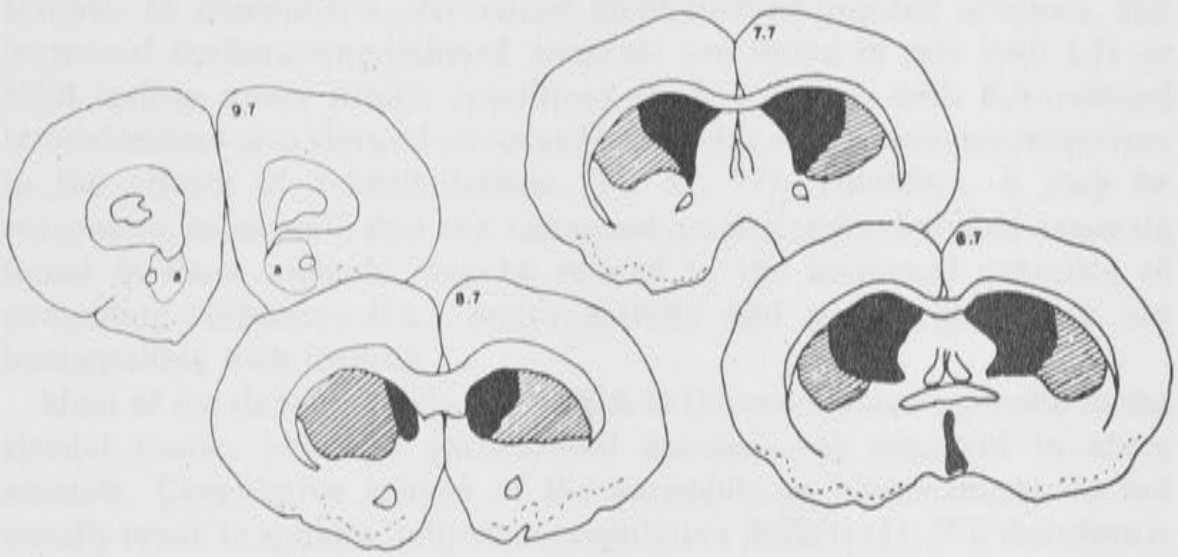

FIG. 8. Pictorial illustration of representative striatal lesion produced by 3 nmol kainic acid in experiments 2 through 4. Numbers correspond to frontal planes of König and Klippel atlas (28).

in rats with striatal lesions ($t(16) = 2.45$, $P < 0.03$ and $t(16) = 2.13$, $P < 0.05$, respectively).

In all KA-treated rats bilateral forebrain ventricular dilation and striatal shrinkage were found. Most of the dorsal striatum was devoid of neuronal cell bodies. The size and stain density of the fiber bundles of the internal capsule in the dorsal striatum were not appreciably altered. The dorsal striatum was densely packed with glial cells which extended slightly into the globus pallidus, although there was no significant reduction in large pallidal neurons. In three brains some cortical encroachment was seen in the deeper layers around the cannula tract, and in two brains the cannula was somewhat ventral causing unilateral cellular loss into the prepyriform cortex and amygdala. In no brains was there any appreciable damage to the lateral hypothalamus or substantia nigra. The lesions produced by KA in these animals were somewhat larger than those seen in experiment 1. In four animals with the largest lesions, loss of neuronal cells was found in the CA3–CA4 layers of the hippocampus, as reported by Wuerthele *et al.* (68). The brains of the control rats showed slight glial infiltration along the cannula tract, but were otherwise intact. Figure 8 shows a representative histological reconstruction of the striatal lesion produced by 3 nmol KA. Detailed representative histological pictures of the resulting damage produced by the present method of KA injection were published elsewhere (32, 35).

Discussion

The results demonstrate that anorexia induced by either *d*-amphetamine or fenfluramine was significantly enhanced in rats with striatal KA-induced

lesions. In comparison, *decreased* amphetamine-induced anorexia and increased fenfluramine-induced anorexia are found in rats with LH or NSB lesions under similar conditions (3, 8, 18). Rats with KA-induced striatal lesions also showed increased locomotor and stereotypic responses to the effects of *d*-amphetamine (34, 35, 57). Therefore, it may be reasonable to assume that the enhanced amphetamine-induced anorexia found in these animals may be related to the increased presence of competing responses (i.e., motor activity and stereotypy) which are incompatible with feeding.

Most of the damage produced by KA in these animals was found in the striatal tissue, although extrastriatal damage was apparent in some animals. Coagulative lesions of the amygdala or hippocampus do not usually result in aphagia, adipsia, or regulatory deficits (13, 65); therefore it is unlikely that damage to these regions could account for the present results. Frontal cortex lesions, however, can produce feeding and drinking deficits similar to those found in the acute stages of the rats with KA-induced striatal lesions (27). The frontal cortex and dorsal striatum, which are linked by the corticostriatal projection (22), were also shown to be functionally related (14). It is possible that the small amount of cortical damage may have contributed to the acute ingestive behavioral deficits reported here; however, the behavioral sequelae in the chronic stage of animals with striatal lesions is different from those observed in rats with cortical lesions (27). It is also possible that the small amount of gliosis in the globus pallidus may have contributed in part to the alterations of feeding and drinking reported here, because it has been shown that electrolytic lesions of this structure can affect these behaviors (43). The possibility that these results could be due to damage in the lateral hypothalamus or substantia nigra is remote; those regions were intact in all brains, and also, there was no change in tyrosine hydroxylase activity in the striatum of the KA-treated rats (experiment 1).

GENERAL DISCUSSION

The present experiments confirm that KA-induced striatal degeneration causes short-term aphagia and adipsia and long-term body weight reductions (48, 55). After recovery, *ad libitum* food and water intake is not reliably different from that of controls. Such regulatory and body weight deficits in the acute state are similar to those found after lesions of the LH (3, 31, 52, 64) or one of its transversing fiber systems, the NSB (1, 8, 18). The intrinsic neurons of the striatum receive their dopaminergic innervation from the substantia nigra via the NSB (41). Therefore, it is reasonable to assume that selective lesions of these neurons, postsynaptic to the dopamine terminals and characterized in part by cholinergic and

GABAergic cells, are also involved in eating and drinking behaviors, as well as the maintenance of normal body weight. Although the deficits in animals with striatal lesions were not as large as those seen after LH and NSB lesions, it should be noted that based on the neurochemical and histological evidence, the lesions produced by 3 nmol KA did not destroy all neurons in the striatum, but instead caused only partial destruction. It appears likely that more extensive lesions of the striatum would produce more drastic and longer-lasting aphagia and adipsia, such as those seen in animals with NSB lesions.

The interpretation of these results depends on the selectivity of destruction of neuronal cell bodies after KA injections. This selective neurotoxic effect of KA has been demonstrated in the striatum (10–12, 15, 32–35, 38, 39, 50, 55–58), hippocampus (62), cerebellum (23), hypothalamus (47), and retina (61) of various species of animals. A number of investigations recently showed a loss of afferent terminals in the striatum after KA injections (7, 9, 42) and have questioned the specificity of KA in destroying only neuronal perikarya. However, it was also shown that many factors can affect the neurotoxicity of intracerebral injections of KA. The dose, volume, infusion rate, and stability of the solution (39); the rat strain (58), and the nature and amount of anesthetic (45) can all affect the action of KA. The investigations that have questioned the specificity of KA in the striatum have used from 4 to 15 times the dose, in at least twice the volume, and with faster infusion rates than those utilized in the present study. These differences could lead to much more severe destruction in the striatum than demonstrated here and may account for the discrepant results obtained by various laboratories.

Pettibone *et al.* (48) suggested that, as with NSB lesions, rats with KA-induced striatal lesions also display a LH-like syndrome. Although in the acute stage this may be the case, the present experiments suggest that the nondopaminergic neurons of the striatum may play a different role in the chronic or "recovered" animals. Recent findings in this laboratory showed that in rats with KA-induced striatal lesions, in which the dorsal striatum is primarily affected, there exists an enhanced dopaminergic activity as indicated by increased nocturnal locomotor activity [(33) and Sanberg, Pisa, and Fibiger, *in preparation*] and enhanced amphetamine-induced locomotor activity (36, 57) and stereotypy (34, 35). It has been speculated that after such lesions an inhibitory striatonigral feedback loop is disrupted causing increased dopaminergic activity in the ventral striatum and nucleus accumbens (32, 34, 35). According to a number of tests in the chronic KA-treated animals, such as increased nonprandial drinking and amphetamine-induced anorexia, the results are opposite to those found in rats with lesions of the dopaminergic NSB. This suggests that in animals with KA-induced lesions, increased dopaminergic function in the ventral

striatum, etc., compared to decreased function in animals with NSB lesions may be responsible for these differences. Concurrently, it would be predicted that KA lesions of the ventral striatum and/or nucleus accumbens would result in chronic feeding and drinking deficits similar in direction to those seen in animals with NSB and LH lesions following electrolytic ventral striatal lesions. Indeed, Neill and Linn (44) demonstrated food and water regulation deficits similar to those in animals with NSB and LH lesions following electrolytic ventral striatal lesions.

"Progressive loss of weight are early symptoms in Huntington's chorea" (6). "Most choreic patients lose a considerable amount of weight as their disease progresses" (2). Although most references to the weight loss associated with Huntington's disease are subjective statements related to clinical findings such as "all patients were nonobese" (51) and those cited above, the mean body weights of Huntington's disease (53 kg) and control (64.5 kg) patients reported in various studies (26, 49) and in a previous study by Oepen (46) based upon findings obtained from 217 postmortem cases support "the striking prevalence of cachexia and marasmus in patients with hereditary chorea" (4). To evaluate objectively the body weight symptoms in Huntington's disease we examined the case histories of 11 nondeceased hospitalized patients and 11 matched (duration of hospitalization, age, diet, and height) non-Huntingtonian control patients (Sanberg and Fibiger, in preparation). This study indicated clearly that loss of body weight was a progressive symptom of Huntington's disease, which can lead to a marasmic condition in the later stages, in spite of being fed double portions of daily meals. Increased caloric expenditure from hyperkinesis may not adequately account for the continuous weight loss in the later stages because it is quite common for the disease to progress toward hypokinesis (the Westphal variant) in the ultimate stages (4). It is evident that the weight loss is not associated with decreased appetite or anorexia since as Bruyn (4) best describes it: "His (the patient's) interest in the world narrows down and simple bodily functions become a major focus of interest. The well-known ravenous appetite of choreic patients bordering on gluttony thus too becomes an understandable phenomenon." The continuous hyperkinesis while the patient is awake would explain the need for more caloric intake.

Kainic acid-induced lesions of the striatum produce an animal syndrome which is similar to Huntington's disease, this being supported by biochemical, histological, psychological, and pharmacological similarities between the two conditions (12, 33–35, 38, 55, 57, 60). In this study and those of others (48, 55) it was demonstrated that these animals also show a marked weight loss which appears to be associated with lesions of the striatum. Therefore, it may be that the loss of weight associated with Huntington's disease is also a result of the pathology associated with the

striatum. This is in contrast to the suggested relationship between this weight loss and the pathology found postmortem in the lateral hypothalamus of brains from Huntington's patients (5). Originally, Facon and his colleagues (17) suggested that the cachexia was a result of degeneration of the dorsal paramedian hypothalamic nucleus. It is also likely, however, that these weight changes reflect multifocal damage occurring in the disease, including in addition to those above, the cortex (4, 54), globus pallidus (4, 29), and subthalamic nucleus (4, 29). These areas have all been shown to affect ingestive behavior in animal studies (27, 43, 44, 65).

It is possible that the prandial drinking results may have some predictive value for Huntington's disease patients and in turn augment the use of this animal model as a tool for studying the pathophysiological conditions of the disease. Thus, it would be of interest to determine if Huntington's patients also increase their water intake during fasting. The present results would suggest that they may. Refsum (53) suggested polydipsia as one of the vegetative symptoms in Huntington's disease (4).

Another similarity to Huntington's disease which was found in these animals was the potentiated response to the increased serotonergic activity caused by fenfluramine (experiment 4). Although the role of serotonin in the disease is not presently clear (2), patients have been reported to show a worsening of symptoms (exaggerated response) when given agents which are believed to enhance central serotonergic activity (2, 66).

Only a few investigators have attempted to increase the weight or have even measured body weight changes of patients with Huntington's disease after administration of various drugs (16, 24, 30). Loss of weight in animals produced by KA-induced lesions of the striatum may be a useful model for evaluating the effectiveness of various experimental agents in restoring the weight to normal values. Recently, muscimol (a GABA agonist) was shown to increase food intake when administered to rats (21). Conceivably drugs such as muscimol, which would tend to restore the lowered GABA activity found both in patients with Huntington's disease (2, 12, 54) and in animals with KA-induced lesions (12, 38, 55, 60), may also reverse the consistent weight loss associated with these two conditions.

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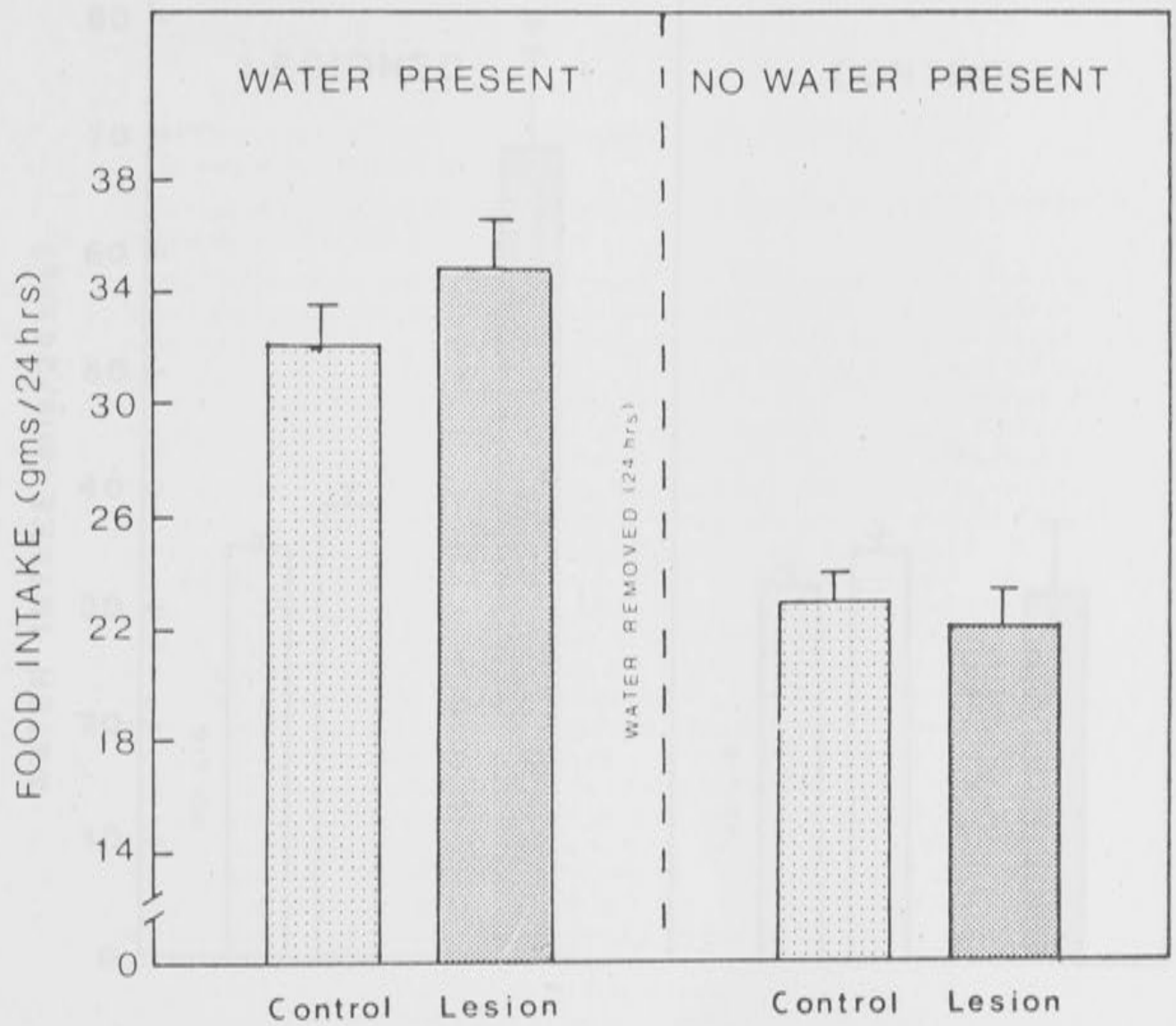

Figure 9. Mean food intake (24h) for control and kainic acid-induced striatal lesioned rats under conditions of available water and no available water. Error bars represent 1SE.

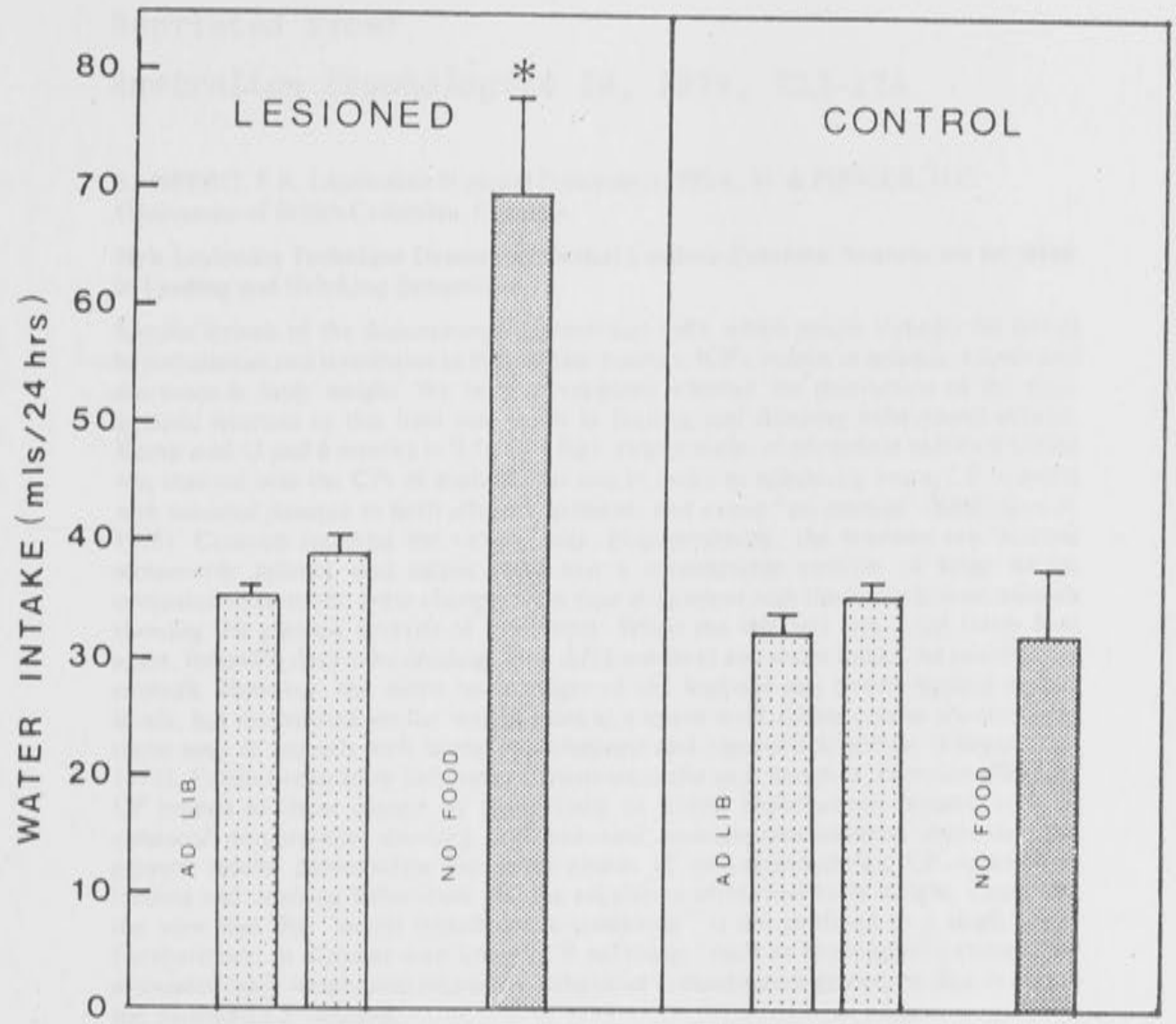

Figure 10. Mean water intake (24h) for 10 control and 10 rats with kainic acid-induced striatal lesions under conditions of available food (AD LIB) and no available food. Error bars represent 1SE.

*$P < 0.001$ (two-tailed test).

Reprinted from:

Australian Psychologist 14, 1979, 223-224

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New Lesioning Technique Demonstrates that Caudate-Putamen Neurons are Involved in Feeding and Drinking Behaviours

Specific lesions of the dopaminergic nigrostriatal tract, which passes through the lateral hypothalamus and terminates in the caudate-putmen (CP), results in aphagia, adipsia and decreases in body weight. We have investigated whether the destruction of the post-synaptic neurons to this tract also result in feeding and drinking behavioural deficits. Kainic acid (3 and 6 nmoles in 0.5μ of 1.0μ1, respectively, of phosphate buffered saline) was injected into the CPs of male Wistar rats in order to selectively lesion CP neurons with minimal damage to both afferent terminals and axons "en passage' (Sanberg *et al*, 1978). Controls received the vehicle only. Postoperatively, the lesioned rats became temporarily aphagic and adipsic, and lost a considerable amount of body weight compared to controls; these changes were dose dependent with the 6 nmole dose animals showing the greatest severity of symptoms. When the lesioned rats could freely feed again, following daily tube-feeding, their *ad libitum* food and water intake did not differ to controls. However, the mean body weight of the lesioned rats never reached control levels, but maintained similar weight gains at a lower level. These results are similar to those seen in animals with lateral hypothalamic and nigrostriatal lesions (Fibiger *et al*, 1973). Further regulatory behaviour experiments showed, however, opposite affects of CP lesions to those caused by nigrostriatal or lateral hypothalamic lesions, such as *enhanced* non-prandial drinking and *increased* amphetamine-induced anorexia. The present results demonstrate the involvement of non-dopaminergic CP neurons in feeding and drinking behaviours and the regulation of normal body weight, supporting the view that the "lateral hypothalamic syndrome" is not confined to a single locus. Furthermore, in diseases with known CP pathology, such as Huntington's chorea, the associated body weight and regulatory behaviour symptomatology may be due in part to the damaged CP neurons.

Fibiger, H.C., Zis, A.P., and McGeer, E.G. Feeding and drinking deficits after 6-hydroxydopamine administration in the rat: Similarities to the lateral hypothalamic syndrome. *Brain Research*, 1973, *55*, 135-148.

Sanberg, P.R., Lehmann, J., and Fibiger, H.C. Impaired learning and memory after kainic lesions of the striatum: a behavioural model of Huntington's disease. *Brain Research*, 1978, *149*, 546-551.

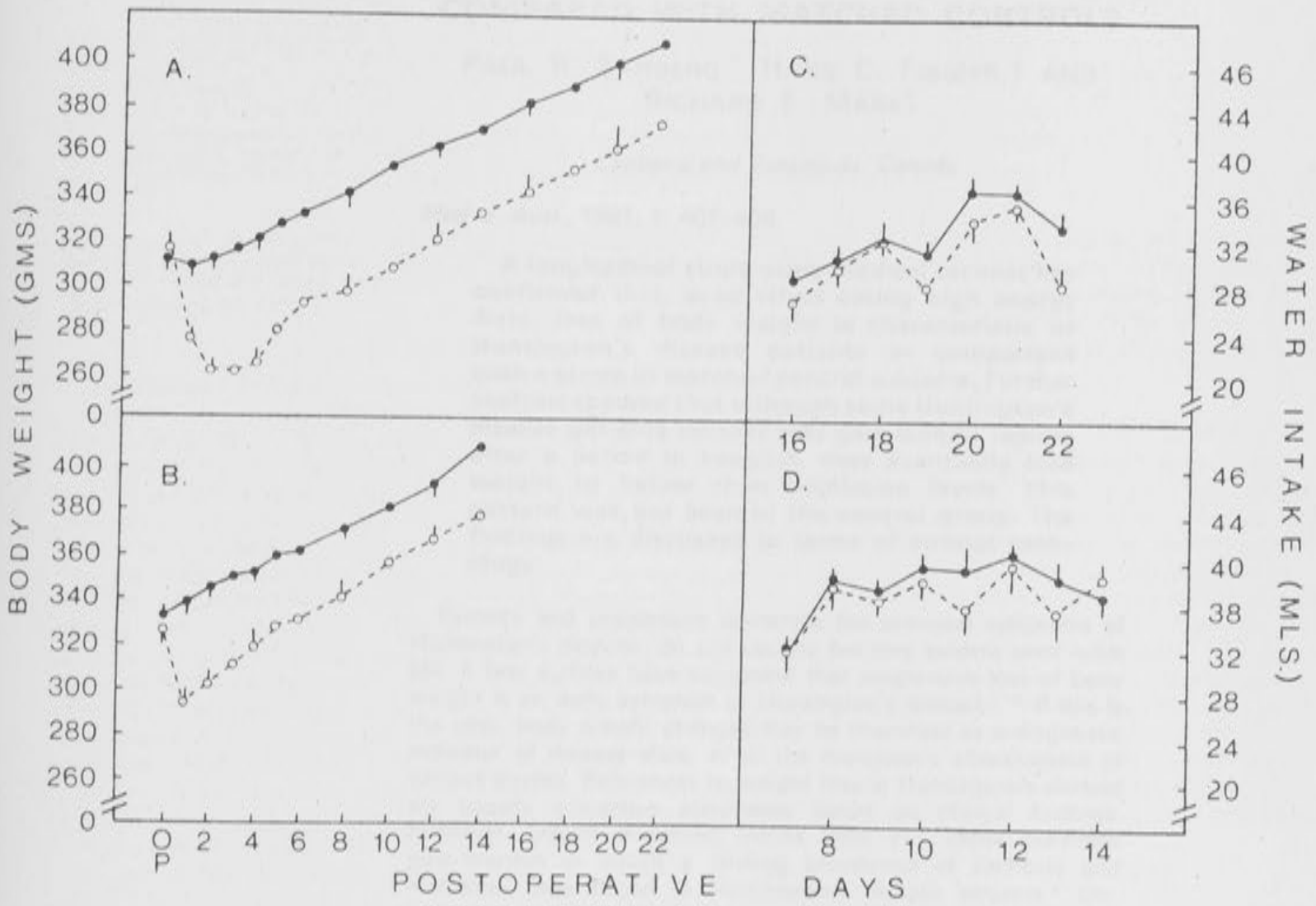

Figure 11. Mean body weights (A and B) and mean water intake (C and D) for rats with kainic acid-induced striatal lesions (open circles) and control (solid circles) rats. Upper (A and C) and lower (B and D) represent rats injected with 6 and 3 nmoles kainic acid, respectively. Error bars represent 1SE.

APRIL 18, 1981

THE MEDICAL JOURNAL OF AUSTRALIA

BODY WEIGHT AND DIETARY FACTORS IN HUNTINGTON'S DISEASE PATIENTS COMPARED WITH MATCHED CONTROLS

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Med. J. Aust., 1981, 1: 407–409.

A longitudinal study using medical records has confirmed that, even when eating high energy diets, loss of body weight is characteristic of Huntington's disease patients in comparison with a group of matched control subjects. Further analysis showed that although some Huntington's disease patients initially may gain weight rapidly after a period in hospital, they eventually lose weight to below their admission levels. This pattern was not seen in the control group. The findings are discussed in terms of striatal pathology.

CHOREA and progressive dementia, the principal symptoms of Huntington's disease, do not usually become evident until adult life. A few authors have suggested that progressive loss of body weight is an early symptom of Huntington's disease.[1-3] If this is the case, body weight changes may be important as a diagnostic indicator of disease state, or of the therapeutic effectiveness of various agents. References to weight loss in Huntington's disease are usually subjective statements based on clinical findings. However, Oepen presented results from 217 cases examined post-mortem in which a striking prevalence of cachexia and marasmus was found in Huntington's disease patients.[4] Unfortunately, there has not been a detailed and controlled study performed on the body weights of such patients over the course of their illness. The present study was undertaken to compare these possible changes in Huntington's disease patients with a matched control group, in an effort to determine if, indeed, body weight loss is symptomatic in Huntington's disease.

PATIENTS AND METHODS

The medical records of seven female and four male patients with Huntington's disease as confirmed by clinical assessment and family histories were used in this study. A control group of 13 patients (five male) matched for sex, age, height, duration of stay in hospital and psychiatric medications was chosen from the same hospital unit (Riverview Hospital, British Columbia, Canada). Ten of the control patients were diagnosed as having organic brain syndromes either with presenile dementia (two patients), brain tumour (two patients), cerebral anoxia (two patients), alcoholism (one patient), brain trauma (one patient), viral encephalitis (one patient) and subarachnoid haemorrhage (one patient). The other three control patients were diagnosed as having alcoholism, schizophrenia or presenile dementia (one patient each). All patients were in hospital continuously from their admission date until the date of this study (September, 1978). Body weight records were analysed only over the period spent in the hospital. For the purpose of analysis, diets were grouped into three categories: general, reducing, and double portions. The general diet contained approximately 9211 kilojoules daily. Food was presented in regular, pureed, minced, high roughage, low residue, high protein or soft forms. The reducing diets were presented in similar forms, but usually consisted of between 5024 kilojoules and 7536 kilojoules. The double portion diet is self-explanatory. Patients that were not able to feed themselves properly were fed by a hospital staff member. There were no reliable differences between groups in the number of times patients were fed by staff members.

All patients were receiving medications for psychiatric disorders during their period in hospital. The drugs were primarily phenothiazines, tran-

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TABLE 1
*Body Weight Changes in Huntington's Disease and Control Patients**

Patient Group	Number of Patients	Age (Years)	Height (cm)	Duration of Stay in Hospital (Years Between Admission and Survey Periods)	Admission Body Weight (kg)	Survey Body Weight (kg)	Survey as Proportion of Admission Body Weight
Control	13	58.0 ± 2.0	166.5 ± 3.1	9.5 ± 1.6	61.5 ± 3.2	59.5 ± 3.4	97.9% ± 5.1
Huntington's disease	11	57.0 ± 2.1	164.2 ± 1.9	9.4 ± 1.7	56.5 ± 3.7	47.1 ± 3.1‡	84.0% ± 3.6†

* Data represent mean ± standard error. Age is expressed as current age, and height is expressed as admission height.
† Significantly different from controls, $P<0.05$.
‡ Significantly different from controls ($P<0.02$) and significantly different from admission weight ($P<0.002$).

quilizers, sedatives and antidepressants. There was no substantial difference between the drugs used by the control patients and Huntington's disease patients. Paired and unpaired Student's *t*-test were used to evaluate the significance of the data.

RESULTS

As depicted in Table 1, there were no significant differences between Huntington's disease and control patients in mean age ($t=0.35$, df=22, $P>0.10$), height ($t=0.64$, df=22, $P>0.10$) or duration of stay in hospital ($t=0.04$, df=22, $P>0.10$). Similarly the mean body weight of both groups at the time of admission did not differ significantly ($t=1.05$, df=22, $P>0.10$). However, mean body weight measured at the end of the period in hospital (survey body weight) was significantly lower in the Huntington's disease group ($t=2.67$, df=22, $P<0.02$). Further analysis of survey mean body weight showed that only the Huntington's disease group significantly dropped in body weight compared with their admission values (Huntington's disease group, $t=4.08$, df=10, $P<0.002$; control group, $t=0.71$, df=12, $P>0.10$), and compared with the control group, as expressed as a proportion of admission body weight ($t=2.16$, df=22, $P<0.05$).

It was found that, after admission, a little over half the patients in each group increased their body weight (Table 2). Analysis of these patients revealed that this mean increase in body weight did not differ significantly between groups ($t=0.97$, df=12, $P>0.10$). However, the duration of time to reach maximum body weight was significantly less in the Huntington's disease group ($t=2.22$, df=12, $P<0.05$). In the patients who gained body weight in hospital, all the Huntington's disease subjects lost weight after reaching their maximum body weight, whereas only 62.5% of the control patients lost weight. It is of interest that the patients in the control group who did not lose weight were all female. No further differences in sex or other discernible characteristics could be found in body weight changes, primarily because of the small number of subjects in each group.

TABLE 2
*Analysis of Huntington's Disease and Control Patients Who Increased Body Weight After Admission**

Patient Group	Proportion of Total Patients	Proportion of Weight Increase	Number of Years to Maximum Weight
Control	55%	16.3% ± 4.4	6.4 ± 1.4
Huntington's disease	62%	23.0% ± 5.4	2.5 ± 0.8†

* Data represent means ± standard error of the mean; this analysis is only of those patients who increased weight after admission.
† Significantly different from controls ($P<0.05$).

At the time of admission there were essentially no differences in the type of diet fed to either group of patients. All the Huntington's disease and 11 of the control patients were on general diets. The other two control patients were fed reducing diets. When the last one-third of the period in hospital was analysed it was found that approximately one-half of the patients in both groups were predominantly on general diets (Table 3). The remaining patients of the control group were on reducing diets and those of the Huntington's disease group were on double portion diets. During this period only one Huntington's disease patient was on a reducing diet (7536 kilojoules). There were no essential differences in the form of the diet presented to each group. It was also found that during the last half of the period in hospital, almost every patient in each group was given pureed, minced or soft forms of their diet.

DISCUSSION

In agreement with previous reports[1-4] the present study indicates clearly that loss of body weight is a progressive and characteristic symptom in most Huntington's disease patients. Furthermore, such body weight loss was not seen in a group of matched control patients with various neurological disorders. Interestingly, it was found that approximately one-half of the patients in both the Huntington's disease and control groups increased body weight after their admission to hospital. This probably reflected poor dietary and eating conditions before being admitted to hospital. While the amount of weight gained did not differ between groups, it appeared that the Huntington's disease patients reached their maximum body weight much sooner than controls. This may be related to an increased appetite since, as Bruyn describes it: "His (the Huntington's disease patient's) interest in the world narrows down and simple bodily functions become a major focus of interest. The well known gluttony thus becomes an understandable phenomenon".[2] Even though some of the Huntington's disease patients rapidly increased body weight after admission they soon lost this and continued to lose weight to below admission levels. This pattern was not seen in the control patients. Similarly, many Huntington's disease patients were fed very high energy diets in an attempt to keep their body weight loss to a minimum. The hyperkinesis while the patient is awake could explain the need for a higher energy intake. Increased energy expenditure from hyperkinesia, however, may not adequately account for the continuous weight loss in the later stages since it is quite common for the disease to progress towards hypokinesis (the Westphal variant),[2] as was seen in some of the present HD patients.

TABLE 3
*Type of Diet Patients Were Predominantly Fed During the Latter Part of the Period in Hospital**

Patient Group	Type of Diet		
	General	Reducing	Double Portion
Control	54%	46%	0%
Huntington's disease	46%	9%	46%

* Data are expressed as a proportion of the total number of patients in the control ($n=13$) and Huntington's disease ($n=11$) groups. The data are taken from approximately the last one-third of the period in hospital for each patient.

Originally, Facon *et alii* suggested that the cachexia observed in Huntington's disease patients was a result of hypothalamic damage, specifically in the dorsal paramedian nucleus.[5] Bruyn, on the other hand, found neuronal loss in the lateral hypothalamus,[6] an area which plays a role in body weight maintenance in animals.[7] It is also likely that these body weight changes reflect multifocal damage occurring in Huntington's disease patients' brains, in-

cluding the cortex,[2 8] globus pallidus, and subthalamic nucleus.[2 9] These areas have all been shown to affect ingestive behaviour in animals.[10-13]

Unfortunately, it was not possible to obtain a comparable group of matched control subjects with other extrapryamidal disorders who resided under the same environmental conditions as the present HD patients. However, it is likely that the differences demonstrated in the HD patients may be true for several disorders of the basal ganglia. The fact that specific disruption of the dopaminergic nigrostriatal tract in rats, which degenerates in Parkinson's disease, severely impairs feeding and body weight regulation, supports this assumption.[14 15]

Recently, it has been shown that kainic acid induced lesions of striatal neurons in rats, which produce remarkable neuropathological and psychopathological similarities to Huntington's disease[16 17] resulted in marked body weight loss.[15 18] Furthermore, this body weight loss is closely related to the amount of striatum damaged.[15] It is possible that the progressive loss of body weight found in Huntington's disease patients may be related to the progressive degeneration occurring in their striatum. However, it is also possible that the mechanisms may involve extraneuronal factors such as hyperpyrexia, food absorption and endocrine changes. For example, increased choreiform activity produced by striatal neuronal degeneration may increase body temperature which, over a long period of time, could result in decreased body weight. This seems unlikely since analysis of body temperature in the present population of patients over the course of the study did not show differences between Huntington's disease and control subjects (Sanberg, unpublished data). In light of the various neuroendocrine changes which have been observed in HD patients,[2 20 21 22] it may be worthwhile evaluating a possible causal relationship between these changes and body weight loss.

Edmonds presented interesting data showing a relationship between dietary conditions in HD patients and death resulting from respiratory disease.[23] He showed that 10 patients with difficulty in swallowing died of bronchopneumonia within about three months after they were changed to a fluid or semifluid diet. Furthermore, he reported two patients having a "ravenous appetite, being excessively greedy and being unclean eaters" who died of asphyxia due to inhalation of food. Edmonds concluded that the "increased incidence of respiratory deaths in long standing cases of Huntington's chorea is probably the result of aspiration of fluids (predisposing to bronchopneumonia) or foods (predisposing to death either from asphyxia or bronchopneumonia), secondary to the involuntary movements affecting swallowing".[23] It is tempting to speculate that the body weight differences between HD and control patients could simply be due to deglutition difficulties. However, this seems unlikely since in the present study assistance in terms of the type of diet and feeding was given to patients in order to keep energy intake as constant as possible. Apparently, though, it is quite important that great care should be taken when feeding HD patients exhibiting dysphagia.

In conclusion, since body weight symptomatology in Huntington's disease may be related to its neuropathology, especially striatal degeneration, it may be an important measure for evaluating the effectiveness of various therapeutic agents, or for estimating the degree of striatal pathology. Only a few investigators have attempted to increase or even measure body weight changes in Huntington's patients after administration of various drugs.[24 25] In view of the present results, body weight changes deserve further attention as possible diagnostic indicators of disease state in Huntington's disease.

ACKNOWLEDGEMENTS

The writers appreciate the excellent assistance of Ms A. Larson and Ms L. McSweeny in collecting the present data and giving advice on matters pertaining to the medical records. Appreciation is also extended to Professor E. D. Bird, Professor E. G. McGeer, Dr I. G. Morgan and Dr W. P. Bellingham for helpful comments and to Ms B. Piper for typing the manuscript. This work was completed in partial fulfilment of the Doctor of Philosophy degree by the first writer.

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THE AVIAN PALEOSTRIATUM AND BODY WEIGHT:

PRELIMINARY FINDINGS

Unpublished.

Compared to the extensive investigations on the neural mechanisms of body weight regulation in mammals (see Sanberg and Fibiger, 1979), practically nothing is known about these mechanisms in birds. Most of the published work has been performed by two separate groups of investigators. Kuenzel and his colleagues have shown that there are two hypothalamic nuclei involved in body weight regulation in the White-throated Sparrow, *Zonotrichia albicollis*. As in rats (Mufson, 1980), they demonstrated that electrolytic lesions of the ventromedial nucleus resulted in hyperphagia and obesity, whereas, bilateral lesions in the lateral hypothalamus caused aphagia and body weight loss (Kuenzel, 1972; Kuenzel and Helms, 1967; Kuenzel and Helms, 1970). In chickens, Lepkovsky and colleagues have also shown aphagia and body weight loss after lesions of the hypothalamus. However, their lesions were not localized to any specific hypothalamic nuclei, except the general "posterior" region (Feldman *et al.*, 1957; Lepkovsky and Yasuka, 1966). The rather sparse available evidence thus suggests that birds have neurological mechanisms controlling body weight similar to those seen in mammals. Because of a possible role of the mammalian striatum in body weight regulation (Sanberg and Fibiger, 1979), body weight data from various striatal manipulative studies in chickens were analysed to see if these areas play a role in avian body weight regulation.

In the first study, cycloheximide, glutamate or saline were injected into the forebrain as described elsewhere (Sanberg *et al.*, 1981). In another study, lesions of the neostriatal and paleostriatal areas in the chick forebrain were produced as described elsewhere (Sanberg and Mark, 1981a). Body weights were measured during the course of other studies on drug effects in the birds and the data was analysed for the present preliminary study.

Table 1 shows the effects of forebrain injections of cycloheximide,

glutamate or saline on the mean body weight of the chickens 2 weeks or 7 weeks later. Both groups given cycloheximide or glutamate showed significantly less body weight than saline-injected controls at 2 weeks of age ($U = 27$, $n_1/n_2 = 10/12$, and $U = 30$, $n_1/n_2 = 11/12$, $ps < 0.05$, respectively). At 7 weeks of age the mean body weights did not differ between groups. However, the variance in the cycloheximide-injected group was significantly larger than the saline-injected group (F ratio = 2.38, df = 20, 19, $p < 0.05$), but not in the glutamate-injected group (F ratio = 1.92, df = 20, 21, $p > 0.05$).

Table 2 shows the results of chickens which received neonatal lesions of the neostriatal or paleostriatal areas. There were no significant differences between birds with lesions of the neostriatum and their control group ($t = 0.37$, df = 16, $p > 0.10$). The body weights of birds with lesions of the paleostriatum and their control group were significantly different ($t = 2,14$, df = 18, $p < 0.05$). The birds with paleostriatal lesions were the only ones in the above studies which would not peck at grain for the first day after treatment. However, within 48h all birds were eating and drinking.

Rogers *et al.* (1974) first reported lowered body weights in 2 week-old chicks injected with cycloheximide on day 2 of life. However, when measured at 24 weeks old, the difference in body weight was no longer present. The findings reported here also showed decreased body weight in cycloheximide-treated and glutamate-treated (these two groups are thought to share a common mechanism, see Sanberg *et al.*, 1981) chickens at 2 weeks of age. By 6 weeks of age, however, this mean difference was not evident, although the cycloheximide-treated group showed significantly greater variance than the control group. Rogers *et al.* (1974) did not report the variances of their 24 week-old birds. The disruption in body weight gains may be due to neurochemical pathology thought to occur in the

Table 1

Body Weights of Chickens Injected with Cycloheximide, Glutamate or Saline when Newly Hatched[†]

Age (approx.)	Saline (n)	Cycloheximide (n)	Glutamate (n)
2 weeks	110.3±2.3 (12)	97.5±4.2[*] (10)	95.3±3.6[*] (11)
6 weeks	305.0±14.5 (20)	300.5±21.8[**] (21)	286.4±15.4 (22)

[†]Data represents means ± standard errors of the means in grams.

[*]Significantly different from saline-injected controls, $p < 0.05$.

[**]Variance is significantly different from saline-injected controls, $p < 0.05$.

Table 2

Body Weights in Chickens (approx. 4 weeks old) Which Received Bilateral Neostriatal or Paleostriatal Lesions When Newly Hatched

Group	Mean Body Weight (n)	Standard error
Control	257.2 (9)	19.1
Neostriatal Lesions	268.3 (9)	23.6
Control	246.0 (10)	13.3
Paleostriatal Lesions	211.5 (10)*	10.7

*Significantly different from control group, $p < 0.05$.

paleostriatum of the cycloheximide or glutamate-injected chickens (Sanberg and Mark, 1981b). However, because of the relatively gross nature of these injections it is also possible that diencephalic damage may have contributed to the results. Olney and Price (1978) have demonstrated that a single systemic injection of glutamate into neonatal mice produces initial weight loss followed by obesity. These injections in mice result in various diencephalic lesions. The significant effect of variance found in older cycloheximide treated birds was a result of animals being very heavy or light for their age. It is possible that such effects are due to different degrees of diencephalic damage. More careful histology correlated with behaviour, than previously reported (Rogers *et al.*, 1974), should be performed on these chickens.

Removal of all forebrain areas dorsal of the laminae medullaris dorsalis, including the neo- and hyperstriatal areas, did not produce any deficits in body weight in chickens. These areas are thought to be homologous to the neocortex of mammals (Karten and Dubbeldam, 1973). In rats, cortical lesions do not produce any gross alterations in body weight (Sanberg, unpublished data). The mammalian striatum may play a role in body weight regulation since kainic acid lesions of this structure resulted in lowered body weights (Sanberg and Fibiger, 1979). Preliminary work with kainic acid lesions of the chicken paleostriatum (the homologue of the mammalian striatum, Karten and Dubbeldam, 1973) reported here demonstrated a significantly long term decrease in body weight in chickens intrapaleostriatally injected with 3nmoles of kainic acid. Therefore, as in mammals, the avian basal ganglia may have a role in body weight regulation. Further work is needed to clarify this.

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CHAPTER FIVE

THE STRIATUM AND DISEASE

Chapter 5.

The Striatum and Disease

This chapter consists of the following parts and appendices:

Part 5.1 Chapter overview

Higher levels of the nervous system frequently have a suppressing influence on the lower levels. A lesion in one area may cause other levels to function in an unrestrained manner. Basal ganglia diseases present many examples of release from higher control (positive signs). In addition to causing release phenomena, central lesions can also directly impair the function of specific nuclei on tracts (negative signs). Positive and negative signs are included in nearly all clinical syndromes due to disease of the basal ganglia. These diseases cause marked abnormalities in motor function, but generally do not cause alterations in muscle strength. The motor dysfunction is characterized by involuntary movement disorders (positive signs) that occur either at rest or with muscle activation and that usually disappear with sleep. Changes in muscle tone and altered postural reflexes (negative signs) can also be present. For many of the disorders of the basal ganglia, only clinical descriptions are available, but in the case of Parkinson's and Huntington's diseases, there has been considerable progress towards understanding the anatomical and biochemical bases of the dysfunctions.

Lesions of the dopamine-containing neurons of the substantia nigra, pars compacta, have been a common finding in Parkinson's disease. Recognition of the degeneration of the dopaminergic nigrostriatal tract, and the profound reduction of the level of striatal dopamine and its metabolite homovanillic

acid has provided the rationale for the successful use of L-dopa in Parkinsonian patients (Hornykiewicz, 1966; Calne and Sandler, 1970). The relatively specific nature of the pathology in Parkinson's disease has allowed the production of many good animal models for investigating intensively the importance of dopaminergic mechanisms in the features of Parkinsonism (Duvoisin, 1976). In particular, the discovery that stereotaxic injections of the neurotoxin, 6-hydroxydopamine could lesion dopamine pathways selectively, has provided important information on the role of the nigrostriatal tract in motor control (Dray, 1979; Duvoisin, 1976).

Huntington's disease is a devastating genetic illness characterized by progressive chorea and dementia. It is thought that the movement disorders in Huntington's disease result from a selective loss of intrinsic striatal neurons, whereas extrinsically originating neurons which traverse or terminate in the striatum are unaffected. Biochemically, dopamine and serotonin, which originate extrastriatally, are relatively normal, whereas acetylcholine and GABA, located in the intrinsic striatal neuronal perikarya, are dramatically decreased (Coyle *et al.*, 1977).

There has not been, until recently, a good animal analogue for Huntington's disease. Most approaches have focussed on hyperkinesias in animals resulting from genetic conditions (Koestner, 1973) and various types of brain lesions (Klawans and Weiner, 1974). Unfortunately, there is no genetic

condition in animals which produces the typical pathology seen in human Huntington's disease, nor until recently has any brain lesion technique been successful in reproducing the specific neuronal losses that occur in the disease. Injection of various transmitter-influencing drugs into the striatum has produced interesting dyskinesias, however, the effects of such treatments are short-lived (see Table 1).

In 1976, Coyle and Schwarcz, and McGeer and McGeer independently took advantage of the neurotoxic effects of glutamate and its analogues to produce neuronal destruction in the rat striatum similar to that found in the brains of Huntington's disease victims. Thus, kainic acid injections destroyed the neuronal perikarya of the striatum, with an associated marked decrease of cholinergic and GABAergic neurochemical markers, and no apparent effect on either the dopaminergic nigro-striatal fibers, the serotonergic raphe-striatal fibers or the glutamatergic cortico-striatal fibers (Coyle *et al.*, 1978). In my Master's Thesis (Sanberg, 1978) the behavioural similarities of rats after striatal injections of kainic acid and human patients with Huntington's disease were studied. These results were published previously (Sanberg *et al.*, 1978, 1979). Since then most of the work I have done on rats has been involved in some way with this 'animal model'. Throughout the previous chapters this has been quite evident. Part 5.2.1 is a short review of this animal model which incorporates much of the research reported in this Thesis. The major contribution this animal model has provided to our understanding Huntington's disease

Table 1.

Drugs Used for Producing Dyskinesias in Animals

Through Intrastriatal Administration*

Dopamine System	Source
Dopamine	Cools & Van Rossum, 1976; Costall & Naylor, 1975
Amphetamine	Cools & Van Rossum, 1976
Apomorphine	Cools & Van Rossum, 1976
3-Methoxytyramine	Dill *et al.* 1976
Acetylcholine System	
Acetylcholine	Dill *et al.* 1968
Carbachol	Dill *et al.* 1968; Murphy & Dill, 1972
Alcuronium	McKenzie & Viik, 1975
GABA System	
Picrotoxin	Pycock, 1976; Standefer & Dill, 1978
Allylglycine	Pycock, 1976
Bicuculline	Standefer & Dill, 1978
D-Tubocurarine	McKenzie & Viik, 1975; McKenzie *et al.* 1972
Thiosemicarbazine	McKenzie & Viik, 1975.
Serotonin System	
5-Hydroxytryptamine	Cools & Van Rossum, 1972

continued on next page

Table 1 (cont.)

Others

Mescaline	Dill, 1972
Somatostatin	Rezek *et al.*, 1977

*Dyskinesias are defined as stereotyped, choreiform, athetoid, hyperkinesia and epileptoid behaviours.

is that the neuropathology may be due to abnormal glutamate neurotransmission. Olney and de Gubareff (1978) have proposed that an adult-onset disturbance in glutamate uptake might underlie the neurodegenerative changes. Evidence in support of abnormal glutamate mechanisms in human Huntingtonian patients has recently been found, in that fibroblasts from patients show degeneration and loss of viability following treatment with glutamate, whereas matched control cultures do not (Gray *et al.*, 1980). At present, research is being performed on all aspects of this hypothesis in order to find possible beneficial treatments for sufferers of the disease. The subsequent four parts of this chapter are concerned with striatal pathology and the role of possible glutamate mechanisms.

In Part 5.2.2 (Sanberg *et al.*, 1979), we injected kainic acid in three strains of rats to see if genetic factors could influence its neurotoxicity. The data showed that the striata of Wistar and Chester Beatty rats are more sensitive to the neurotoxic effects of kainic acid than those of Sprague-Dawley rats. In another study there were no differences between male and female littermate rats in the ability of kainic acid to destroy cholinergic neurons in the striatum (Part 5.2.3). Thus, while genetic differences may play a role on the magnitude of the resulting biochemical alterations, the factor of sex does not appear to be relevant. This is interesting in view of the fact that tardive dyskinesia has been reported to be more frequent in elderly females and that extrogen enhances striatal dopamine receptor sensitivity (Bedard *et al.*, 1979).

Recently, it has been demonstrated that dopamine receptors localized on the glutamatergic cortico-striatal terminals (Schwarcz *et al.*, 1978) have an inhibitory role on the release of glutamate (Mitchell and Doggett, 1980; Rowlands and Roberts, 1980). The neurotoxic effects of kainic acid on striatal neurons are dependent on the presence of this pathway (Bizierre and Coyle, 1978; McGeer *et al.*, 1978). In order to determine if the effects of this neurotoxin are dependent on a release of glutamate *per se*, as has been suggested, we have studied the effects of large doses of bromocriptine, a dopamine agonist which should reduce glutamate release, on kainic acid neurotoxicity in the striatum (Part 5.2.4, Sanberg and Creese, 1981). Bromocriptine did not affect kainic acid striatal neurotoxicity. Providing that inhibition of glutamate release was achieved in the rats, as it is *in vitro*, the results support the view (McGeer *et al.*, 1978b) that glutamatergic neurotransmission *per se* is not involved in KA neurotoxicity, and that only the presence of the glutamatergic terminal is required, in some way, for kainic acid to exert its effects. An interesting result of this study was a surprisingly long term inhibition of choline acetyltransferase activity by bromocriptine. This is consistent with the inhibitory action of dopamine on cholinergic neurons in the striatum (Stoof *et al.*, 1979) and with recent findings that bromocriptine is an irreversible dopamine agonist (Bannon *et al.*, 1980).

Van Gelder (1978) has shown that taurine regulates glutamate retention in neuronal structures, thereby influencing the tissue content of glutamate and of GABA. Concurrently, Muramatsu *et al.* (1978)

demonstrated that taurine can suppress the stimulated release of acetylcholine from neurons. Because of these effects of taurine and the recent report that it improves Huntington's disease symptomatology (Agnoli *et al.*, 1977) we thought it worthwhile to examine the possible effects of chronic treatment with taurine on the neurotoxic action of kainic acid in rat striatum (Sanberg *et al.*, 1979, Part 5.2.5). In two experiments (Part 5.2.5), taurine had an apparent protection effect on cholinergic neurons against the neurotoxic actions of kainic acid. This was consistent with previous data indicating that taurine may have some membrane stabilizing properties (Muramatsu *et al.*, 1978). The effect was, however, very slight, suggesting that kainic acid injections cause an excitation so powerful that it can be affected only slightly by taurine treatment. It is possible that taurine may have a larger therapeutic effect on the relatively slower striatal neuronal degeneration found in Huntington's disease; providing that the degeneration is 'excitotoxic' in nature (Coyle *et al.*, 1978).

Taurine is no longer considered to be an 'inert' amino acid in the central nervous system (Barbeau *et al.*, 1975). In addition to the role mentioned above, taurine has been suggested to play a role in other diseases, including epilepsy (Van Gelder, 1978) and hereditary mental depression (Perry, 1976). Because of the interest in taurine and various diseases, we thought it was of interest to study the effects of taurine on behaviour. Appendices 11, 12 and 13 (Sanberg and Ossenkopp, 1977; Sanberg and Fibiger, 1979; Sanberg and Willow, 1980, respectively), report the actions of taurine on motor (Appendix 11), psychological

(Appendix 12) and convulsive (Appendix 13) behaviour. A figure is included at the end of Appendix 13 which gives a diagrammatical representation of the results on locomotor activity mentioned in that paper. Taurine was shown to inhibit some types of motor activity (Sanberg and Ossenkopp, 1977) and impair the acquisition and retention of a step-down passive-avoidance task (Sanberg and Fibiger, 1979) in normal rats. Furthermore, we have confirmed that taurine does act as an anticonvulsant (Barbeau *et al.*, 1975), although its effective dose curve is limited on hypoxic convulsions, and it is not as effective as a standard anticonvulsant, phenobarbitone (Sanberg and Willow, 1980). This is probably why clinical trials of taurine have been very limited over the last few years (Van Gelder, 1978).

In Chapter 2, the actions of various psychotropic drugs on rats with striatal lesions induced by kainic acid were studied. Part 5.2.1 reviews the effects of these drugs. It appears that the animal model may be beneficial for screening possible therapeutic agents in Huntington's disease. Furthermore, the animal model may provide some information relevant to various controversies surrounding the disease such as the contribution of striatal pathology in the motor and behavioural symptomatology. Another controversy concerns anaesthesia in Huntingtonian patients. An enhanced sensitivity to anaesthetic drugs in Huntington's disease patients was reported by Davies (1966) and Gualandi and Bonfanti (1968), but, Farina and Rauscher (1977) were unable to confirm this. Using the kainic acid animal analogue of Huntington's disease, we were interested in determining how responsive these animals were

to pentobarbitone anaesthesia. The results of Part 5.2.6 (Sanberg *et al.*, 1981a) demonstrate that rats with kainic acid-induced striatal lesions show an enhanced sensitivity to the motor inhibiting effects of pentobarbitone, but not to the inhibitory effects on corneal or pain reflexes. These findings thus suggest that Huntington's disease patients may have enhanced sensitivity to the motor inhibitory effects of sodium pentobarbitone, but not to the anaesthetic effects of this drug. On anaesthetizing patients with these or similar disorders it could be misleading to interpret an abnormally fast induction of motor depression as an index of increased sensitivity to the anaesthetic effects of sodium pentobarbitone. It may be possible to resolve the conflicts in the literature in these terms.

It has been quite evident throughout this Thesis that the biochemical, anatomical and behavioural sequelae of kainic acid injections into the striatum of rats appear similar to those of Huntington's disease (for review see Part 5.2.1 and Coyle *et al.*, 1978). Based on these results and the fact that the striatum receives a major glutamatergic pathway from the neocortex, it has been proposed that excessive glutamate neurotransmission may offer an explanation for the degeneration that occurs in patients with Huntington's disease (Part 5.2.1, Coyle *et al.*, 1978). This excitotoxic hypothesis of the pathogenesis of Huntington's disease also leads to a possible explanation of tardive dyskinesia, which occurs after long-term therapy with dopaminergic blockers. Part 5.2.7 (Sanberg and Mark, 1980) is a letter explaining this relationship and warning

of possible hazards of treating tardive dyskinesia with monosodium glutamate, as suggested by Holland (1979). Treatment with neuroleptics removes the presumed inhibition of the dopaminergic nigrostriatal pathway onto the striatal cholinergic interneurons. In addition, neuroleptics may block presynaptic dopaminergic inhibition located on the terminals of the glutamatergic corticostriatal pathway (Mitchell and Doggett, 1980; Rowlands and Roberts, 1980). Thus, the lack of this inhibition could cause an otherwise normal excitatory glutamatergic input from the cortex to become relatively excessive and to gradually destroy striatal interneurons. This would result in a gradual shift in the dopamine-acetylcholine balance in the striatum, a biochemical condition thought to underlie involuntary movements (de Silva, 1977).

In addition to the excitotoxic hypothesis of Huntington's disease (Part 5.2.1 and Coyle *et al.*, 1978), another theory suggests that the disease is autoimmune in nature. McMenemy (1961) first reported the presence of a possible immunological reaction in Huntington's disease. Husby *et al.* (1977) reported the presence of an anticaudate neuronal antibody and Barkley *et al.* (1977) showed that a cellular immune response of lymphocytes exists in the presence of brain tissue in patients with Huntington's disease. It is possible that corticosteroids, by reducing production of abnormal antibodies, might bring about a favourable therapeutic response. In Appendix 14 (Brown *et al.*, 1979) we report two cases of Huntington's disease patients who showed unfavourable responses when treated with the corticosteroid, prednisone. Although these results do not support the autoimmune

theory for Huntington's disease, the possibility still deserves pursuit, possibly with less advance cases. If part of the chronic degeneration process is due to antibodies, corticosteroid treatment might inhibit progression of the disease, but it would be unlikely to induce any dramatic reversal of established symptomatology.

Birds and Basal Ganglia Disease

The use of birds in the study of basal ganglia disease is practically non-existent. This is because mammals, especially rats, which are closer to the human in striatal organisation, afford a good subject of investigation for this purpose. However, Rieke (1980) has reported that kainic acid-induced lesions of the paleostriatum in pigeons produce movement disorders. Histologically, Rieke (1980) found that large neurons in the paleostriatum primitivum and nucleus intrapeduncularis (homologues of the globus pallidus) were destroyed by kainic acid. In addition, small neurons in the paleostriatum augmentatum (homologue of the caudate-putamen) were destroyed. An interesting finding was that there was virtually no damage of structures outside the paleostriatum. Thus, olfactory cortex, hippocampus, lobus parolfactorius, septal nuclei and archistriatum were not affected. Many of these areas are damaged in rats following intrastriatal injections of kainic acid (Sanberg *et al.*, 1979) presumably a result of the seizure effects of the drug (Ben-Ari *et al.*, 1978, 1980). Behaviourally, injected birds showed irregular, jerky movements of the head. These movements included shaking or snapping

the head toward the side of the injection. Some birds demonstrated episodes of rapid turning to the ipsilateral side. These effects were noticed by 24h after injection. Unfortunately, no mention of behaviour after 48h post-injection is mentioned, therefore it is not known whether the abnormal involuntary movements were permanent effects. Rieke (1980) reported no convulsive activity following injections of kainic acid.

My preliminary work on kainic acid injections in the paleostriatum of chicks has also demonstrated a lack of convulsive behaviour in these birds. Immediately after emerging from anaesthesia the birds showed intense locomotor activity in bilaterally injected chicks, and turning behaviour in the ipsilateral direction in chicks with unilateral injections. This intense activity stopped by 6h after injection. At 12h the birds began to show irregular jerks of the head and neck. The birds showed no obvious signs of flacidity. Wings were held in a normal resting position and walking was typically normal. However, the movement disorders were completely gone within 36h. Over the next four weeks, until used for the haloperidol study reported in Chapter 2, the birds appeared normal.

The fact that paleostriatal injections of kainic acid in birds do not produce seizure activity and extrapaleostriatal damage is of interest. In rats injections of kainic acid into the striatum usually induce intense convulsive behaviour after coming out of anaesthesia, and extrastriatal pathology, which can be reduced by treatment with anticonvulsants (Ben-Ari *et al.*, 1978, 1980; Sanberg *et al.*, 1979; Zaczek *et al.*, 1978).

Further study should be undertaken in birds to determine the basis of this interesting difference between birds and mammals. It is of interest that the chicks which received large forebrain injections of glutamate in the studies reported elsewhere (Sanberg *et al.*, 1981, Chapter 3) all showed intense temporary convulsive behaviour immediately after injection. Therefore, excitotoxic compounds can produce convulsions in birds.

Kainic acid injections into the striatum of rats appear to produce a good model for studying various aspects of Huntington's disease (Part 5.2.1). However, Huntington's disease is characterised by damage to cortical, striatal and pallidal areas. In fact, Coyle and his colleagues (1977) suggested that, in addition to injections in the striatum, kainic acid injections in the cortex and globus pallidus of rats would also be necessary to produce a close analogue of the disease. The proximity of the neostriatum ('neocortex'), paleostriatum augmentatum ('striatum') and paleostriatum primitivum ('globus pallidus') to each other in the bird telencephalon means that single injections of kainic acid may reach all areas. Thus, providing that these areas are truly homologous to corresponding areas in mammals, the use of birds in studying basal ganglia disorders may provide interesting results. However, based on the vast amount of knowledge being found with rats, it is likely that, at the present time, research into basal ganglia function in non-human primates may provide the most realistic answers to questions raised in human disorders of the basal ganglia. Perhaps the major advantage of chickens is

that they are extremely inexpensive and easy to handle in large numbers, which makes them quite attractive for many purposes in the present economic climate, even though they are quite distant in evolutionary terms from humans.

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DUPLICATION OF NEURAL AND BEHAVIOURAL PATHOLOGY OF HUNTINGTON'S DISEASE IN ANIMALS: A NEW METHOD USING A SINGLE INTRASTRIATAL INJECTION OF KAINATE.

Presented at the

1st Australian Huntington's Disease Association Conference,

Melbourne, 1979.

Submitted for Publication to

Medical Journal of Australia

as "Glutamate and Huntington's Disease".

(with G.A.R. Johnston)

ABSTRACT

Very small injections of kainate, an analogue of the neurotransmitter glutamate, into rat neostriatum, have been shown to duplicate remarkably well neural and behavioural pathology seen in Huntington's disease (HD). This experimental procedure provides an excellent model system for investigating the pathogenesis and treatment of HD. Work with the animal model has suggested that abnormal glutamate neurotransmission may underlie the neuropathology found in HD. At present, research is being performed on all aspects of this hypothesis in order to find possible beneficial treatments for sufferers of the disease.

Huntington's disease (HD) is a progressive genetic disorder involving neuronal degeneration of discrete areas of the CNS, the basal ganglia being among the most severely affected. Psychological and motor disorders are the primary symptoms of HD. Because the nature of HD is still little understood, it would be important to reproduce aspects of the disease in laboratory animals in order to study the possible mechanisms involved in HD neuro- and psychopathology, and to provide a screening test for possible therapeutic agents.

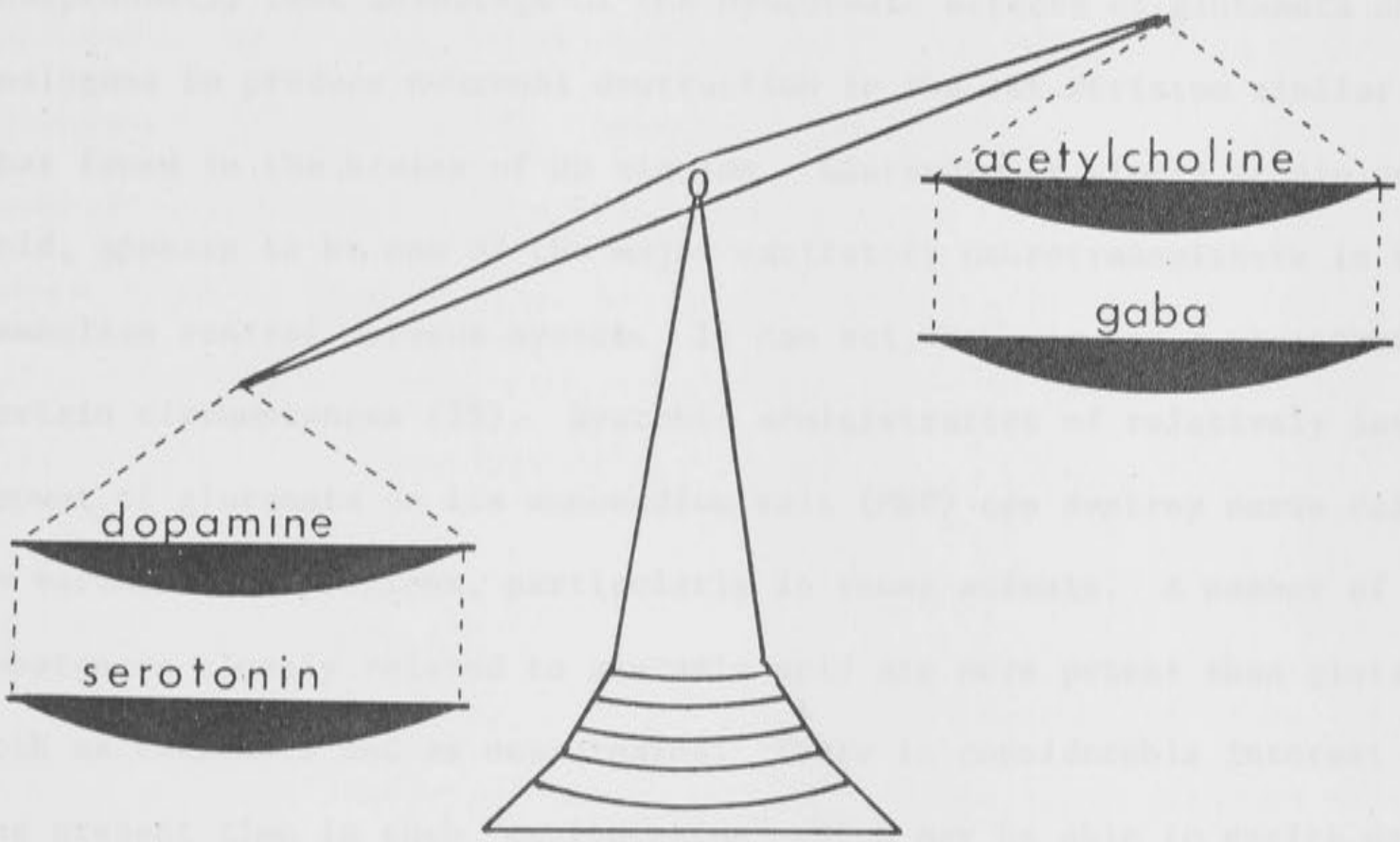
Previously, many directions have been explored to discover an appropriate animal syndrome. Most approaches, however, have focussed on hyperkinesias in animals resulting from genetic conditions (for review see 32) and various types of brain lesions (for review see 30). Unfortunately, there is no genetic condition in animals which even remotely resembles the pathology seen in human HD, nor until recently has any brain lesion technique been successful in reproducing the specific neuronal losses that occur in HD. The major obstacle with these studies is that the movement disorders in HD result from a selective loss of intrinsic striatal neurons, whereas extrinsically originating neurons which transverse or terminate in the striatum are unaffected. This selective neuronal loss results in a functional imbalance between various neurotransmitter systems in the striatum (Figure 1). The major neurochemical findings have shown that dopamine and serotonin which originate extrastriatally are relatively normal, whereas acetylcholine and GABA located in striatal neuronal perikarya are dramatically decreased (11).

In the strictest sense, an animal model which duplicates successfully the neural and behavioural pathology of HD should satisfy these four criteria:

1) the animals should show neuropathological changes similar to those seen in HD,

Figure 1

Striatal Neurotransmitter Balance in Huntington's Disease



2) motor and behavioural symptomatology of a nature parallel to HD symptoms should be represented,

3) responsiveness to psychotropic drugs in a manner similar to that observed in patients with HD should be demonstrated,

4) there should be a genetic component involved.

In 1976, Coyle and Schwarcz (10) and McGeer and McGeer (41) independently took advantage of the neurotoxic effects of glutamate and its analogues to produce neuronal destruction in the rat striatum similar to that found in the brains of HD victims. Glutamate, a simple acidic amino acid, appears to be one of the major excitatory neurotransmitters in the mammalian central nervous system. It can act, however, as a neurotoxin under certain circumstances (25). Systemic administration of relatively large amount of glutamate as its monosodium salt (MSG) can destroy nerve cells in various brain regions, particularly in young animals. A number of substances closely related to glutamic acid are more potent than glutamate both as excitants and as neurotoxins. There is considerable interest at the present time in such "excitotoxins" which may be able to excite neurones to death. The neurotoxic properties of kainate in particular are being used extensively in neurobiology to produce selective neuronal lesions. Kainate, a conformationally restricted analogue of glutamate, appears to activate a particular population of postsynaptic receptors for glutamate (26) of which a number of different receptor populations have been found in the brain.

Over the past few years a large amount of research correlating the changes that occur in kainate-lesioned (KAL) rats and HD have been performed. At present there is good evidence to suggest that the KAL model satisfies all the aforementioned criteria. Furthermore, this model which reproduces the specific neuronal destruction that occurs in HD, has offered some important clues about the underlying causes of this disease.

Neuropathology of KAL rats

Microinjections of nanomolar quantities of kainate into the striatum result in degeneration of local intrinsic neurons, leaving apparently intact both axons and terminals of extrinsic neurons. The resulting biochemical and histological picture appears strikingly analogous to that described in the striatum of HD patients upon post-mortem examination (10, 41). The neuropathological sequelae of intrastriatal KA injections have been reviewed extensively (11, 9). Table 1 summarises and compares the biochemical and morphological changes occurring in the striatum of HD and KAL rats. A recent study by Krammer (33) has revealed a characteristic pattern of striatal neuronal degeneration and sparing which is extremely similar to that observed in post-mortem HD striatum.

Symptomatology of KAL rats

Although chorea, as such, is not recognizable in KAL rats, they do show an abnormal locomotion which is characterised by an increase in swing time and a decrease in stance time compared to control rats. Thus, the lesioned rats put their paws on the ground for shorter periods of time and swing them longer than normal rats. This has been suggested to be analogous to the locomotion pattern observed in patients with HD (23). Another similarity to HD which exists in the motor activity of KAL rats is that, compared to controls, their locomotor activity is markedly potentiated during arousal (38). HD patients also show much greater choreiform movements during their awake period and when aroused (67).

Although the psychological and psychiatric symptoms of HD have been attributed to cortical atrophia, recent studies with KAL rats have revealed marked impairments in learning, memory and affect without any concomitant cortical damage. Table 2 summarises the psychological deficits observed in KAL rats. Recent neuropsychological studies have shown that HD dementia

TABLE 1: A summary of biochemical and morphological changes in the striatum reported in rats intrastriatally injected with kainate (KAL), and Huntington's Disease (HD).

Neuronal Index	KAL	HD
BIOCHEMICAL INDICES*		
GABA[a,b,c,d,e]	Decreased (1)	Decreased (2)
Acetylcholine[a,b,c,d]	Decreased	Decreased
Dopamine[a,b,c,d]	Normal or Increased	Normal or Increased
Serotonin[b,c,d]	Normal	Normal
Angiotensin[a]	Decreased (3)	Decreased
Enkephalin[b]	Decreased	Decreased
RECEPTOR BINDING		
GABA	Decreased (72)	Decreased
Muscarinic	Decreased	Decreased
Serotonin	Decreased	Decreased
Dopamine	Decreased (18, 66)	Decreased
MORPHOLOGY		
Intrinsic neurons	Decreased	Decreased
Glia	Increased	Normal or Increased
Afferent terminals	Normal	Normal
Internal capsule	Normal	Normal
Mass	Decreased	Decreased
Ventricles	Dilated	Dilated

*Biochemical indices used for KAL striatum are:

a: synthetic enzyme activity
b: transmitter level
c: transmitter uptake
d: transmitter release
e: transmitter metabolite.

Numbers in parentheses are references in addition to the review by Coyle *et al.*, (12).

TABLE 2: A summary of psychological deficits reported in rats intrastriatally injected with kainate.

Behavioural Task	
Impaired acquisition and retention of passive avoidance	(49, 58, 61)
Impaired acquisition and resistance to extinction of one-way avoidance	(49)
Resistance to extinction of bar pressing learning	(61)
Impaired acquisition and retention of spatial alternation	(17, 51)
Impaired retention of delayed alternation	(16)
Deficit in short-term memory of reward-alternation runway task	(50)
Failure to spontaneously initiate activity in spontaneous alternation task	(60)
Enhanced neophobia in alleyway maze	(60)
Decreased reflexive freezing to electric shock	(61)

Numbers in parentheses refer to references.

is different from those dementias associated solely with cortical pathology and may be a result of "subcortical" pathology. In addition, the term "subcortical dementia" has become synonomous with HD dementia (46). The psychological impairments observed in KAL rats suggest that striatal pathology may underly such impairments in HD.

Marked body weight decreases and regulatory deficits which are symptomatic in HD patients (57) have also been shown to occur in KAL rats (56). Furthermore, motor impairments in KAL rats produce difficulty in eating and handling small food pellets.

Response to psychotropic drugs in KAL rats

In order to use an animal model to screen possible therapeutic agents in Huntington's disease, it would first be important to show that the animals respond to various psychotropic agents of known effects in a similar manner to HD patients. The most common pharmacotherapy for HD is based on the proposed neurotransmitter balance system in HD (Figure 1). Thus, agents which put more weight on the dopamine side and cause further imbalance, tend to worsen the symptoms of HD. Conversely, agents which tend to restore the balance to normal are beneficial. When the effects of such agents were evaluated in KAL rats, behavioural responses similar in nature to that reported in HD patients were found (Table 3). Furthermore, the paradoxical effect of the dopamine agonist apomorphine in failing to exacerbate chorea in HD, was also found in motor behaviour of KAL rats (40, 59).

Genetic influences in KAL rats

KAL rats, of course, are not produced through genetic means, however, it has been demonstrated that some rat strains are more susceptible to kainic acid-induced degeneration of striatal neurons (63).

TABLE 3: Comparison of motor response to various pharmacological agents in rats intrastriatally injected with kainate (KAL) with patients with Huntington's Disease (HD)*.

Drugs used and their relation to neurotransmitter system.	KAL	HD
INCREASED DOPAMINERGIC ACTIVITY		
d-Amphetamine	potentiated (39, 40, 61)	exacerbated (31)
Apomorphine (high dose)	normal (40)	normal (34)
(low dose)	normal (59)	beneficial (8)
DECREASED DOPAMINERGIC ACTIVITY		
Pimozide	normal (38)	slightly beneficial (34)
Haloperidol	decreased (62)	slightly beneficial (68)
DECREASED CHOLINERGIC ACTIVITY		
Scopolamine	potentiated (62)	ND
Benzotropine	ND	exacerbated (29)
INCREASED CHOLINERGIC ACTIVITY		
Pilocarpine	decreased (62)	ND
Physostigmine	ND	slightly beneficial (14)
INCREASED SEROTONERGIC ACTIVITY		
Fenfluramine†	potentiated (56)	ND
Imipramine	ND	potentiated (71)

*The effects on striatal KA animals are expressed in terms of effects on control animals. For HD the effects are expressed in terms of amount of chorea.

†Determined on anorexic activity.

ND: not determined.

Numbers in parentheses refer to the references.

Another important finding has revealed that older rats are also more susceptible to kainic acid (20). Thus, it appears that as in human HD, age and genetic makeup are important factors in KAL rats.

KAL animal model relevance to HD

The single intrastriatal injection of kainic acid into the striatum of rats appears to duplicate remarkably well the neural and behavioural pathology of HD. The KAL model offers a unique opportunity for testing possible pharmacotherapies on this global pathology. Thus, Schwarcz *et al.* (65) demonstrated that γ-acetylenic GABA can completely correct the GABA deficiency in the striatum of KAL rats. Similarly, London and Coyle (36) found that choline chloride, or phystostigmine can at least partially, correct acetylcholine deficits found in KAL rats. It would be important to see if γ-acetylenic GABA and/or choline chloride or phystostigmine also proved beneficial on the behavioural pathology seen in these animals.

The most important contribution of the KAL model to basic research on HD is the fact that a toxic analogue of glutamate or glutamate itself can cause these changes in rats. Therefore, the disorder may be related to some aspect of glutamatergic neurotransmission. The striatum receives a major glutamatergic pathway from the neocortex (53). This pathway has been shown to be intimately involved in the ability of kainate to destroy striatal neurons (7, 44). Thus, removal of the glutamate afferent input to the striatum drastically reduced kainate's neurotoxicity. McGeer and McGeer (41) proposed that overactive glutamate-releasing neurones may underlie the neuronal degeneration found in Huntington's disease. If this were true it may be possible to prevent progression of such degeneration by blocking the postsynaptic action of glutamate with suitable antagonists, or by blocking the release of

glutamate from presynaptic terminals.

Glutamate receptor antagonists

Many workers have been searching for selective glutamate receptor antagonists in order to further investigate the role of glutamate and related compounds in the brain. Recently, Davies *et al.* (13) developed a series of antagonists based on D-α-aminoadipate, which show selectivity for aspartate-activated receptors rather than those activated predominantly by glutamate. Olney *et al.* (48) used DL-α-aminoadipate to block the neurotoxic action of N-methyl-DL-aspartate in mice. Agents showing some selectivity for glutamate-preferring receptors include L-glutamate diethylester and the lotus alkaloid nuciferine. It may be possible to develop therapeutically useful drugs from these receptor antagonists, and aporphine alkaloids related to nuciferine particularly merit closer investigation in this context.

Inhibitors of glutamate release

Release is one of the most difficult synaptic processes to study in the central nervous system and very little is known about the pharmacology of glutamate release. The most interesting drug at present is baclofen, β-(p-chlorphenyl)-GABA, which is used clinically in the treatment of spasticity. Originally developed as a lipophilic derivative of the inhibitory transmitter GABA, baclofen appears to inhibit the release of excitatory transmitters from primary afferent terminals in the spinal cord. In neurochemical experiments baclofen has been shown to inhibit glutamate release *in vitro* from the cerebral cortex (52). It would appear worthwhile to examine baclofen and related compounds as possible therapeutic agents to prevent progression of neurodegeneration in diseases such as HD.

Glutamate uptake

The postsynaptic excitation produced by synaptically released glutamate may be normally terminated by active transport systems which avidly take up glutamate into various cellular compartments. Studies on cats have shown that inhibitors of glutamate uptake markedly potentiate excitation produced by glutamate (35). Olney and de Gubareff (47) have proposed that an adult-onset disturbance in glutamate uptake processes might underlie the neurodegenerative changes in Huntington's disease. Thus it may be possible to diagnose Huntington's disease at an early stage by looking for abnormalities in glutamate uptake processes. Perhaps this might be done by examining easily accessible material such as blood platelets which possess glutamate uptake processes remarkably similar to those in the central nervous system (37). Procedures for the investigation of glutamate uptake processes on various tissue preparations are being developed.

If glutamate does play a major role in Huntington's disease as seems very likely, then pharmacological manipulation of glutamate synaptic mechanisms may be very useful in preventing or slowing down the progression of the disease. Furthermore, abnormalities in glutamate uptake systems might enable early diagnosis of the disease.

The KAL model also allows for the evaluation of the ability of various pharmacological agents to affect the actual kainate-induced striatal neuronal degeneration. McGeer and McGeer (42) found significant protective effects on KA-induced striatal degeneration with a variety of possible glutamate antagonists. Sanberg *et al.* (64) demonstrated that taurine offered some protection to the cholinergic neurons from kainate neurotoxicity. Naloxone also decreases its neurotoxicity on both cholinergic and GABAergic neurons (45), whereas morphine (45) and ethanol (43) enhance KA's neurotoxicity.

Glutamate and early HD symptomatology

It is generally believed that chorea is a result of excessive dopaminergic regulation in the striatum of HD patients (4). While direct evidence for an excess of striatal dopamine has not at present been clearly demonstrated (6), the altered dopamine/GABA-acetylcholine ratio found in these brains (Figure 1) is considered to underlie the abnormal movements (4, 15). However, in the early stages of HD, a time when little if any striatal pathology is present (30), chorea and dementia may be quite pronounced. The glutamate hypothesis may offer a clue about this discrepancy. Recently, Roberts and Anderson (54) demonstrated that glutamate produced a marked stimulation of dopamine release in the striatum of rats, which was abolished following kainate lesions. Thus, it may be worth speculating that in the early stages of HD, increased glutamatergic regulation in the cortico-striatal tract results in excessive dopamine release in the striatum, producing chorea. In the latter stages, however, when glutamate is ineffective in releasing dopamine because of striatal neuronal destruction, the abnormal neurotransmitter balance may play a more major role in maintaining the dyskinesias. It is interesting that in the later stages of the disease, hypokinesis is quite common.

Recent interest has been focussed on the finding of increased dopamine in the nucleus accumbens of HD brains (70) and possible roles for this finding in the psychosis and hyperkinesias of HD patients has been suggested (40, 70). Glutamate has also been shown to have a marked releasing action on dopamine in this nucleus (54).

To speculate even further, it has been shown that intracerebral injection of non-toxic amounts of glutamate produces quite profound learning, memory and perceptual disorders (19, 21). Thus it seems possible that an increase in glutamate neurotransmission through some mechanism in Huntington's disease can cause characteristic symptoms

early in the disease.

Glutamate and Human HD

Only a few studies have been performed to look for abnormal glutamate mechanisms in HD. Kim *et al.* (27) have shown a marked decrease in the amount of glutamate in the cerebrospinal fluid in HD patients. The significance of this at present, however, is not known. Mangano and Schwarcz (37) have presented preliminary evidence on glutamate uptake into platelets of HD patients which indicated a higher affinity in the patient population. More impressively, Gray *et al.* (22) found that HD fibroplasts show degeneration and loss of viability following treatment with glutamate, whereas matched control cultures do not. This is the first evidence of a sensitivity to the toxic effects of glutamate in HD patients.

An Endogenous Kainate-like Material?

Recent studies have demonstrated that KA may activate a particular population of glutamate receptors in the brain. Since KA has not been demonstrated in the vertebrate central nervous system, this specificity suggests that there may be a natural ligand for the "KA receptor". Skerritt and Johnston (69) have found endogenous inhibitors of KA binding which increase with age. These endogenous substances could represent natural ligands for KA receptors. If there were abnormal increases in a natural ligand which was also an endogenous inhibitor for KA receptors in the HD brain, this would help explain previous results showing decreased KA receptor binding in HD post-mortem striatal tissue (23). Thus, it would be quite significant if an endogenous ligand of KA would be found in the brain. In fact, Ruck and colleagues (55) recently reported that methyltetrahydrofolate, the predominant form of folate in

the blood and cerebrospinal fluid, interacts specifically with KA receptors on neuronal membranes. It would be important to determine if methyltetrahydrofolate can cause KA-like neuronal degeneration and if so, whether there is an excess of methyltetrahydrofolate in HD patients.

Concluding Remarks

Only future research will verify if glutamate or an endogenous kainate-like material play a role in HD. The present evidence with the KA animal model and the preliminary results in HD patients strongly supports such a possibility. Therefore, we suggest that all therapies and diets which involve increasing glutamate (5) and perhaps folate in patients be discontinued as a matter of precaution.

The kainic acid animal model has opened many possible avenues of research into HD. Breakthroughs in presymptomatic detection, prophylactic and symptomatic treatments may not be that far off. One exciting line of research has demonstrated the viability of transplanting neonatal striatal tissue into KA-lesioned striata (28). Providing that these transplanted animals show recovery of behavioural function, then it may be that we are one step closer to keeping patients with HD in the families where they belong.

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Strain differences and kainic acid neurotoxicity

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(Accepted December 21st, 1978)

Since Olney's original report[10] on the specific toxic effects of kainic acid (KA) on neuronal perikarya, several studies have been conducted in different laboratories on the anatomical[1–3,5,16] and biochemical[1,6,7,12,15] effects of KA injections into the neostriatum (CP). Much of the variability in the magnitude of the resulting lesions can be undoubtedly attributed to the use of different doses, volumes and rates of injections of the KA solution. It has in fact been shown that each of these injection parameters can remarkably affect the extent of neuronal loss in the CP[7]. Recent observations, however, suggested that a genetic factor may also influence the neurotoxic effectiveness of KA. Thus, it was noted that identical neostriatal injections resulted in a more severe aphagia and adipsia, and in a higher mortality rate in hooded than in Wistar rats (Sanberg and Corcoran, unpublished data). It also appeared that different litters of rats of the same Wistar strain might show considerable differences in the extent of CP damage after identical injections of KA (Pisa, Sanberg and Fibiger, unpublished data). Also, investigators using Wistar rats[3,5–7] have reported CP lesions of similar extent to that found by other investigators using Sprague–Dawley rats[1,2,15,16], even though widely different injection parameters were used.

Here we report biochemical data indicating that strain differences in sensitivity to KA are indeed another factor to be considered in comparing data of various laboratories. Male Wistar albino rats (Woodlyn Farms, Guelph, Ontario), Sprague–Dawley albino rats (Biobreeding Labs, Ottawa, Ontario), and Chester–Beatty hooded rats (Biobreeding Labs, Ottawa, Ontario) weighing approximately 300 g at the time of surgery were included in the study. After anesthesia with pentobarbital (50 mg/kg i.p.) the rats were given unilateral intrastriatal injections of either 2.5 or 5.0 nmol of KA (Sigma Chemical Co.) dissolved in 1.0 μl of phosphate buffered saline solution, pH 7.2, through a 34 gauge cannula over a 5-min period. The cannula was left in place for a further 1 min to allow diffusion of the drug solution. With the incisor bar at 4.2 mm below the interaural line, coordinates used were AP +9.6 and DV +4.5, with reference to the interaural line; and ML —2.8, with reference to the sagittal suture. The rats were operated in two series and an interval of either 10 or 18 days separated surgery from sacrifice. Rats were killed by cervical fracture, and the CP was bilaterally dissected, weighed, and homogenized in 10 vol of either 0.25 M or (for uptake assays)

TABLE I

Neurochemical parameters in the control, noninjected neostriatum of rats of 3 different strains

The contralateral side was injected with either 2.5 or 5 nmol of kainic acid at least 10 days prior to sacrifice. Values represent means ± S.E.M. Protein content was measured on a wet tissue weight basis. Enzymes and glutamate uptake were measured on a protein content basis.

Dose	*Strain*		
	Sprague-Dawley (n)	*Wistar (n)*	*Chester-Beatty (n)*
2.5 nmole KA			
Protein mg/g	10.54 ± 0.20 (10)	10.52 ± 0.28 (10)	10.25 ± 0.26 (5)
CAT*,**	31.28 ± 0.36 (10)	34.96 ± 1.66 (10)	35.21 ± 0.96 (5)
GAD*,**	12.89 ± 0.59 (10)	14.68 ± 0.26 (10)	13.58 ± 0.24 (5)
Glutamate uptake**	3012 ± 204 (5)	2999 ± 202 (5)	—
5.0 nmole KA			
Protein mg/g	9.85 ± 0.35 (8)	10.52 ± 0.42 (10)	10.68 ± 0.30 (4)
CAT*,**	31.33 ± 0.57 (8)	32.99 ± 1.45 (10)	34.66 ± 0.93 (4)
GAD*,**	13.48 ± 0.41 (8)	14.83 ± 0.36 (10)	13.46 ± 0.36 (4)
Glutamate uptake**	2761 ± 152 (3)	3067 ± 154 (5)	—

* Significant strain differences, $P < 0.01$.

** in μmol·h^{-1}·100 mg·protein^{-1}.

0.32 M of sucrose. The activities of the two neurotransmitter synthesizing enzymes choline acetyltransferase (CAT) and glutamic acid decarboxylase (GAD), commonly considered as functional indicators of cholinergic and GABAergic neurons respectively, were measured by previously reported radioenzymatic assay methods[8]. Protein content was measured according to Lowry et al[4]. In the rats of the second series high affinity glutamate uptake, a functional indicator of presumably glutamatergic corticostriatal afferent terminals[9], was also measured as previously reported[9], using samples of a homogenate of the P_2 pellet fraction and sodium-free blanks.

Since the trend of the results was similar in both series, the data of the two series combined are reported. For rats of the Chester–Beatty hooded strain only data from the first series are available, since 8 out of 10 rats operated in the second series died within a few days after surgery. Data were analyzed with two-way analyses of variance, with Strains and Dose as main Factors, using an unweighted means solution. Multiple comparisons between strains were conducted using Scheffé's F test. Since this test is more rigorous than other methods of multiple comparisons a 10% significance level was accepted[14].

Data for non-injected CP are shown in Table I. There were no significant effects of strains on either protein content, $F < 1$, or glutamate uptake, $F < 1$. However, rats of different strains significantly differed in both CAT and GAD activities, $F = 4.4$ and 8.6 respectively, df = 2,44; $P < 0.01$. Multiple comparisons between strains revealed that Sprague–Dawley rats had significantly less CAT activity than either Wistar or Chester–Beatty rats. Also, both Sprague–Dawley and Chester–Beatty rats had less GAD activity than Wistar rats. Neither the main effect of dose nor the interaction term were significant for any of the indices measured, F's < 1.

TABLE II

Neurochemical parameters in the kainic acid injected neostriatum of rats of 3 different strains

Values are expressed as mean percentages ± S.E.M. of the related values in the contralateral noninjected neostriatum.

Dose	*Strain*		
	Sprague–Dawley (n)	*Wistar (n)*	*Chester–Beatty (n)*
2.5 nmole KA			
Protein mg/g	101.8 ± 3.6 (10)	96.8 ± 4.0 (10)	101.7 ± 5.5 (5)
CAT**,***	73.9 ± 2.9 (10)	59.7 ± 2.1 (10)	54.6 ± 1.9 (5)
GAD**,***	75.9 ± 3.1 (10)	61.4 ± 2.3 (10)	57.7 ± 1.6 (5)
Glutamate uptake	88.2 ± 6.2 (5)	95.8 ± 7.83 (5)	—
5.0 nmole KA			
Protein mg/g*	97.6 ± 4.9 (8)	85.9 ± 3.5 (10)	104.7 ± 1.0 (4)
CAT**,***	59.3 ± 3.7 (8)	40.2 ± 3.6 (10)	47.9 ± 1.3 (4)
GAD**,***	65.1 ± 3.2 (8)	47.9 ± 3.3 (10)	51.8 ± 3.3 (4)
Glutamate uptake	102.0 ± 2.2 (3)	103.3 ± 1.8 (5)	—

* significant strain differences, $P < 0.05$.
** significant strain differences, $P < 0.005$.
*** significantly different from lower dose, $P < 0.005$.

Table II shows data in the injected CP, expressed as percentages of the related values in the noninjected CP. Analysis of variance of the protein data revealed a significant effect of strain, $F = 3.8$; df = 2,41; $p < 0.05$. Multiple comparisons between strains indicated a significantly greater decrease of protein content in the Wistar than in the Chester–Beatty rats. No other comparison attained the 10% level of significance. Both the main effect of dose and the interaction term were not significant, F's < 1. There were no significant differences in protein content between the injected CP and their corresponding control CP in either strain. The difference in the injected CP protein content between the Wistar strain and the Chester–Beatty strain rats may reflect greater edema in the injected CP of the Wistar rats during the acute stages following the KA injections.

There were no reliable differences between Sprague–Dawley and Wistar rats in glutamate uptake in the injected CP, $F < 1$, at either dose, $F = 3.07$; df = 1,14. Indeed, in agreement with previous findings[7], KA injections did not affect glutamate uptake, as shown by the lack of substantial differences between control and injected CP in uptake values.

As expected[1,5–7,12,15], the rats of all groups revealed a marked decrease in both CAT and GAD activities in the injected CP. The main finding was a significant effect of strain on the decrease of both CAT and GAD activities, $F = 18.9$ and 18.7 respectively, df = 2,41; $P < 0.0005$. Multiple comparisons between strains indicated that this effect mainly resulted from a significantly smaller decrease of both enzymes in Sprague–Dawley rats compared with rats of the other two strains. Both CAT and GAD decreased significantly more in the rats treated with the higher dose of KA,

$F = 32.7$ and 17.6 respectively, df = 2,41, $P < 0.0005$, with no significant interaction of strain with dose for either measure.

It should be noted that the enzymatic activities were measured on a protein basis. Because of the relatively low protein content found in the injected CP of Wistar rats, enzyme differences between Sprague–Dawley and Wistar rats would have been even more marked if the data were expressed relative to tissue weight, rather than protein content. In fact, no reliable differences in tissue weight were found between control and injected CP in either strain.

It is apparent that in addition to the methods used to inject KA[7] into the neostriatum, genetic differences also play a role on the magnitude of the resulting biochemical alterations. This genetic factor should be carefully considered in quantitative comparisons of the effects of KA injections reported by different laboratories. The present finding of genetic influences on the neurodegenerative effects of KA extend the previously reported biochemical[1,6,7,12,15], histological[2,3,5,16], pharmacological[5,13], and behavioral[3,11,12] similarities between striatal lesions resulting from KA in animals and those resulting from Huntington's disease in man[1].

The present data show that the neostriatum of Wistar rats is more sensitive to the neurotoxic effects of KA than that of Sprague–Dawley rats. On the other hand, Chester–Beatty hooded rats did not substantially differ in this respect, inspite of previous and present observations indicating especially marked behavioral and regulatory disorders, and an unusually high mortality rate in hooded rats after KA intrastriatal injections. The mechanisms responsible for the latter effects in Chester–Beatty hooded rats remain to be studied.

Strain effects were also found on the enzymatic activities of the non-injected neostriatum. It is unlikely that these differences resulted from spread of KA to the control neostriatum of the Sprague–Dawley and the Chester–Beatty rats, since there was no significant effect of dose on the enzymatic activities. To our knowledge this is the first report of strain differences in neostriatal CAT and GAD activities. Whether these differences are related to the effect of strain on the neurotoxic effectiveness of KA remains to be determined.

This work was supported by grants from the Medical Research Council of Canada and the Huntington's Chorea Foundation. We wish to express our sincerest thanks to K. Singh, U. Scherer and D. Money for excellent technical assistance, and Drs. M. E. Corcoran and H. C. Fibiger for helpful comments during the course of this study.

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LACK OF SEX DIFFERENCES IN

STRIATAL KAINIC ACID NEUROTOXICITY

unpublished

Since the original reports by McGeer and McGeer (14), and Coyle and Schwarcz (5) on the specific neurotoxic effects of kainic acid (KA) on neuronal perikarya in the striatum, many studies have been conducted in different laboratories on the anatomical, biochemical and behavioural aspects of these lesions. There are striking similarities between KA-induced striatal lesions and those seen in Huntington's disease (HD). Because this experimental procedure provides a model system for investigating the pathogenesis and treatment of HD, it is important to determine the important parameters governing the variability in the magnitude of the KA-induced lesions. It is known that the use of different doses, volumes and rates of injections of the KA solution can significantly affect the extent of the lesion (15), as can the batch of KA (7), the anaesthetic (23) and the animal strain (22).

There appears to be no substantial sex differences in the incidence or onset of HD (4). However, neuroleptic-induced tardive dyskinesia has been reported to be more frequent in elderly females (2). This can be explained in terms of the recent demonstration that estrogen treatment in rats enhances dopamine receptor sensitivity in the striatum (2,9). Recently, it has been demonstrated that dopamine receptors localized on the glutamatergic cortico-striatal terminals have an inhibitory role on the release of glutamate (17,18). The ability of KA to lesion striatal neurons is dependent on this glutamatergic cortical afferent pathway (3,16). If estrogen increases the sensitivity of striatal presynaptic dopamine receptors then it is possible that KA may have reduced effectiveness in female rats, due to reduced glutamate output. Here we investigated sex differences in sensitivity to KA in the striatum, since many investigators have used rats of either sex.

Male and female (n = 9/group) Wistar hooded rats from the Department of Psychology animal colony, A.N.U., were used. Animals were littermates and

housed in groups of five. At the time of surgery the mean body weights were 353.8g ± 13.7 (S.E.M.) and 229.1 ± 7.41 for male and female rats, respectively. After anaesthesia with pentobarbital (40 mg/kg) the rats were given unilateral intrastriatal injections of 6 nmol KA (Sigma Chemical Co., Lot 117C-0044) dissolved in 1.0 µl isotonic saline solution, buffered to pH 7.2 with 25% NaOH, through a 30 gauge cannula over a five min period. The cannula was left in place for a further 5 min to allow diffusion of the drug solution. The cannula was stereotaxically inserted into the striatum at coordinates corresponding to AP =8.4, DV = 0.8 and ML = 2.2 of the König and Klippel (10) atlas. The rats were weighed daily for seven days after which they were killed by decapitation, and the striatum was bilaterally dissected, weighed and homogenized in 1 ml of distilled H_2O. The activities of the neurotransmitter synthesizing enzyme choline acetyltransferase (ChAc), which is considered as a functional marker of cholinergic neurons was measured as previously described (8). Protein content was measured according to Lowry *et al* (12).

Following KA injections, male and female rats did not differ in the amount of aphagia, adipsia or body weight loss shown over the recovery period (19). The maximum mean body weight loss in males and females was $33.3g \pm 3.6_{\wedge}^{(9\%)}$ and $25.6g \pm 2.8_{\wedge}^{(11\%)}$, respectively, which occurred on day 2 following surgery. Mean body weights returned to preoperative values at the time of decapitation. The ChAc results are reported in Table 1. Both male and female groups showed significant decreases in ChAc activity in the KA-injected striatum compared to their control striatum ($t = 3.82$ and 3.74, respectively, $df = 8$, $p < 0.005$). There were no significant differences between male and female groups in control values, KA-injected values, or the mean percent difference between control and KA-injected values. Protein and wet weight measures did not significantly differ between groups.

Table 1

Choline acetyltransferase activity in control and kainic acid (KA) injected striata of male and female rats.*

Sex (n)	Control Striatum	KA-injected Striatum	% Control
Male (9)	25.68±3.83	13.98±1.89**	58.70±8.78
Female (9)	30.42±4.06	18.79±2.08**	67.36±9.66

*Values are expressed as means±S.E.M. in $nmol \cdot h^{-1} \cdot mg\ protein^{-1}$.

**Significantly different from controls, $p < 0.005$.

It is apparent that sex differences in littermate populations of rats do not influence the magnitude of the resulting biochemical or body weight alterations from instrastriatal KA. If estrogens do influence KA neurotoxicity, levels in a normal population of female rats do not seem to have any effect. The possibility that abnormal estrogen manipulations can change the degree to which the striatum is sensitive to KA remains to be studied. The present finding of a lack of sex differences on the neurodegenerative and body weight effects of KA extend the previously reported biochemical (3,5,13-16, 19,21,22), histological (5,7,14,19), pharmacological (13,19,21) and behavioural (19,21) similarities between striatal lesions resulting from KA in animals and those observed in HD in humans (4,20). Similarly, sex differences are not found in other disease associated with chorea, such as systemic lupus erythematosus and hypoparathyroidism (6). Whether the increased frequency of tardive dyskinesia (2) and the rare cases of chorea associated with pregnancy (11) or estrogen treatment (1) in women are associated with differences in striatal neuropathology remains to be clarified.

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DOPAMINE RECEPTOR STIMULATION AND STRIATAL KAINIC ACID NEUROTOXICITY

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to be published in

Journal of Pharmacy and Pharmacology

Kainic acid (KA) has been shown to have specific neurotoxic effects on neuronal perikarya in the striatum which show striking similarities to striatal pathology seen in Huntington's disease (Coyle et al, 1978). This model system has been useful for investigating the pathogenesis and treatment of Huntington's disease (Sanberg and Johnston, 1981), as well as increasing our knowledge of the functions of the striatum. Recently, it has been demonstrated that dopamine receptors localized on the glutamatergic cortico-striatal terminals (Schwarcz et al, 1978) have an inhibitory role on the release of glutamate (Mitchell and Doggett, 1980, Rowlands and Roberts, 1980). The neurotoxic effects of KA on striatal neurons are dependent on the presence of this pathway. In order to determine if the effects of this neurotoxin are dependent on a release of glutamate *per se*, as has been suggested, we have studied the effects of large doses of bromocriptine, a dopamine agonist which should reduce glutamate release, on KA neurotoxicity in the striatum.

Male 150g albino Wistar rats (Charles River) were injected with 20 mg/kg bromocriptine (gift of Sandoz) in saline vehicle. Control animals received vehicle injections only. Two hours later all rats were given unilateral intrastriatal injections of 3 or 6 nmol KA as described previously (Sanberg et al, 1979), with the exception that halothane anaesthesia was employed. Two hours after surgery, another injection of bromocriptine (10 mg/kg) or vehicle was given to the appropriate animals. Three weeks later the animals were killed by decapitation and the striatum was bilaterally dissected, weighed, frozen and stored at $-20^{\circ}C$. Subsequently the activities of the acetylcholine synthesizing enzyme choline acetyltransferase, which is considered as a functional marker of cholinergic neurons, was measured as previously described (McGeer and McGeer, 1976).

The results are depicted in Table 1. All groups showed significant decreases in choline acetyltransferase activity in the KA-lesioned striatum

Table 1

The effect of bromocriptine on choline acetyltransferase activity in control and kainic acid lesioned striata.[ψ]

Dose (n)	Control Striata	KA-lesioned Striata	% Control
3 nmol KA			
Vehicle (4)	2.21±0.17	1.15±0.36*	52.2±16.2
Bromocriptine (4)	1.36±0.12**	0.75±0.27*	55.3±19.6
6 nmol KA			
Vehicle (4)	1.55±0.22	0.30±0.13*	19.5± 8.3
Bromocriptine (4)	0.97±0.08**	0.30±0.16*	31.0±16.1

[ψ]Values are expressed as means ± S.E.M. in nmoles/mg wet wt/15'

*Significantly different from control striatum, $p < 0.05$ (Student's t test).

**Significantly different from vehicle-treated group, $p < 0.05$.

compared to their matched controls. The 6 nmol groups showed larger decreases than the 3 nmol groups. The percent difference between KA-injected and control striatum did not differ between bromocriptine and control treated groups at either dose of KA. However, bromocriptine did produce significant decreases in control striatal activities of choline acetyltransferase.

The surprisingly long term inhibition of choline acetyltransferase activity by bromocriptine may be consistent with the inhibitory action of dopamine on cholinergic neurons in the striatum (Stoof et al, 1979), and with recent findings that bromocriptine is an irreversible dopamine agonist (Bannon et al, 1980). This inhibition could be produced by a direct post-synaptic agonist action of bromocriptine on dopamine receptors localized on intrinsic cholinergic neurons. Alternatively, bromocriptine may be having a pre-synaptic agonist action on dopamine receptors localized on glutamatergic cortico-striatal terminals (Schwarcz et al, 1978). Recently it was shown that activation of these pre-synaptic dopamine receptors inhibits glutamate release from the cortico-striatal pathway (Mitchell and Doggett, 1980; Rowlands and Roberts, 1980). Thus, a reduced release of this excitatory neurotransmitter in synapses localized on striatal cholinergic neurons could result in reduced cholinergic activity. The lack of effect of bromocriptine on KA striatal neurotoxicity suggests that the former explanation may be correct. The ability of KA to lesion striatal neurons is dependent on the glutamatergic cortical afferent pathway (McGeer et al, 1978a). Therefore, a lack of effect of bromocriptine on KA neurotoxicity may mean that a significant reduction in glutamatergic neurotransmission in bromocriptine-treated rats was not obtained. However, the fact that lower doses of dopamine agonists can markedly reduce glutamate release from cortico-striatal fibers (Mitchell and Doggett, 1980; Rowlands and Roberts, 1980), suggests that inhibition of glutamate was achieved in the present animals. Therefore, the present results support the view (McGeer et al, 1978b) that glutamatergic

neurotransmission per se is not involved in KA neurotoxicity and that only the presence of the glutamate pre-synapse is required in some way for KA to exert its effects.

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Chronic taurine effects on various neurochemical indices in control and kainic acid-lesioned neostriatum

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(Accepted October 12th, 1978)

It is well demonstrated that iontophoretically applied taurine exerts a depressing effect on neuronal firing and the amino acid has been suggested as an inhibitory neurotransmitter, neuromodulator or stabilizer of membrane excitability[2]. Recently, van Gelder[16] revealed evidence showing that taurine regulates glutamic acid retention in neuronal structures, thereby influencing the tissue content of glutamic acid and of λ-aminobutyric acid (GABA). Concurrently, Muramatsu et al.[10] demonstrated that taurine can suppress the stimulated release of acetylcholine from neurons, presumably by stabilizing excitable membranes and thereby suppressing the release of acetylcholine at synapses.

Kainic acid, a rigid glutamate analog with potent excitatory effects on neurons, causes degeneration of neurons when injected locally into the brain, presumably as a result of sustained excitation or depolarization[11]. Injections into the neostriatum of rats cause biochemical, histological and behavioral changes similar to those seen in Huntington's disease[3,6,15]. Because of taurine's known stabilizing effects on excitable membranes and the recent report that it improves Huntington's disease symptomatology[1], it seemed worthwhile to examine the possible effects of chronic treatment with taurine on the neurotoxic action of kainic acid in rat neostriatum. The extent of neuronal destruction was assessed by measuring glutamic acid decarboxylase (GAD) and choline acetyltransferase (CAT) activities in controls and injected striata. Decreases in these enzymes following kainic acid injections have been repeatedly shown to be not only dose-related but correlated with other indices of GABAnergic and cholinergic neuronal loss. The experiments were also designed to determine possible effects on various neostriatal enzyme activities of chronic taurine treatment. These enzymes were GAD, CAT, glutaminase, tyrosine hydroxylase (TH) and cysteine-sulfinic acid decarboxylase (CSAD) which is considered to be a key enzyme in the synthesis of taurine. Oral administration of taurine was used since it has been demonstrated that taurine administered by this route enters the CNS[4,13,14] and because such administration has been used clinically in some neuropathological conditions[2,13,16].

Twenty-four male Wistar rats (Woodlyn Farms, Guelph, Ontario) in total were used in two experimental series, with 12 rats in each group. They were maintained in single cages with free access to food (Purina rat chow) and water (tap water) and a 12-h light–dark cycle. One-half of the rats in each group received chronically administered oral taurine (Fischer Scientific) by the addition of the amino acid (0.9% final concentration, 0.07 M) to the drinking water. Preliminary investigations, as well as reports from Persinger et al[14], indicated that this concentration did not significantly alter fluid consumption. Control rats received tap water. Fluid intake was measured daily and water bottles were refilled about every 4 days. It has previously been shown[4] that taurine is stable in tap water solution. Rats received oral taurine for 22 (group I) or 34 days (group II) prior to surgery. At the time of surgery rats in group I weighed about 500 g and those in group II about 390 g. All animals received unilateral stereotaxic injections of 3 nmol of kainic acid into the neostriatum. The kainic acid was dissolved in 1 μl of phosphate-buffered saline, pH 7.2, and injected through a 34-gauge cannula over a 5-min period. After the injection, the cannula was left in place for a further 5 min before withdrawal. The appropriate coordinates were determined by dye injections in animals of comparable weight. For group I, the coordinates from interaural line and incisor bar at —4.2 were AP 10.6, ML —2.8, DV 4.5 and for group II they were AP 10.1, ML 2.8, DV 4.5.

Experimental rats were kept on oral taurine for approximately one week following surgery. All rats were then sacrificed by cervical fracture, their brains rapidly removed and the neostriata dissected. The tissue samples were weighed and homogenized in 10 vols of cold 0.25 M sucrose. Samples of the homogenates were used for the determination of protein, CAT, GAD and TH by previously reported methods[5,9]. TH assays were done with exogenous cofactor (dimethyltetrahydropteridine). Glutaminase was measured by a radioactive procedure using [U-^{14}]L-glutamine as substrate and a coupled reaction with bacterial GAD[7]. CSAD was assayed using a modification of the method of Pasantes-Morales et al.[12].

Kainic acid was obtained from Sigma Chemical and the radioactive substrates from New England Nuclear except for [1-^{14}C]cysteinesulfinic acid which came from the Commissariat a L'Énergie Atomique, Service de Molécules Marquées, France.

Chronic treatment with taurine under the conditions used did not significantly affect the weight of the striatal samples on the uninjected side, the protein content or any of the enzymic activities measured (Table I). As previously reported, in rats sacrificed approximately one week after injections of 3 nmol of kainic acid into the neostriata, the protein contents, weights and TH activities of the injected striata are not significantly different from those on the contralateral side (Table II). Activities of GAD, CAT and glutaminase, on the other hand, are significantly decreased (Table II)[7,8]. The results in the animals chronically treated with taurine are not significantly different from those in control rats except for the data on CAT activities, where the chronic taurine treatment seemed to give a small amount of protection (Table II). The fact that this possible protective action is only seen with CAT is consistent with previous findings that the effect of kainic acid on CAT activity, and presumably therefore on cholinergic neurons, is somewhat more vulnerable to manipulation by

TABLE I

Weights, protein content, glutamate uptake and some enzyme levels in the neostriata of taurine-treated rats and controls

Data represents mean ± S.D. N = 6 in each group. * Enzyme activities are expressed per hour and 100 mg of protein with GAD, CAT, CSAD and glutaminase being in μmol. TH is in nmol. Glutamate uptake on the P_2 fraction is in μmol/5 min-mg protein.

	Taurine-treated		*Controls*	
	Group I	*Group II*	*Group I*	*Group II*
Weights, mg	40.90 ± 2.28	43.00 ± 1.40	40.40 ± 1.59	40.50 ± 2.64
Protein, mg/g	11.78 ± 0.24	10.86 ± 0.82	11.49 ± 0.45	10.86 ± 0.64
GAD*	15.04 ± 2.04	14.40 ± 0.85	15.25 ± 1.15	13.37 ± 1.82
CAT*	36.93 ± 1.92	37.20 ± 1.72	36.84 ± 1.67	36.87 ± 2.24
TH*	163 ± 14.7	162 ± 9.40	169 ± 11.0	167 ± 7.70
CSAD*	6.34 ± 0.90	6.80 ± 1.10	6.80 ± 0.34	5.75 ± 1.53
Glutaminase*	404 ± 17	428 ± 16	399 ± 20	415 ± 30
Glutamate uptake*	2.77 ± 0.19	2.69 ± 0.25	2.72 ± 0.11	2.67 ± 0.32

TABLE II

Protein, CAT, GAD, TH and glutaminase levels in kainic acid-injected neostriata as per cent of contralateral control

Data represents mean ± S.D. N = 6 in each group.

	Group I		*Group II*	
	Taurine	*Control*	*Taurine*	*Control*
Protein	99.45 ± 3.16	94.83 ± 3.98	93.68 ± 6.27	95.67 ± 3.71
GAD	56.93 ± 5.74	54.82 ± 3.29	47.83 ± 5.53	45.83 ± 4.13
CAT	58.89 ± 4.54*	43.52 ± 4.66	50.67 ± 5.38*	36.33 ± 2.92
TH	98.41 ± 5.72	109.10 ± 4.84	94.90 ± 4.85	98.19 ± 7.25
Glutaminase	70.47 ± 3.24	69.23 ± 3.47	63.48 ± 6.00	61.16 ± 2.06
Weights	109.00 ± 6.40	98.40 ± 2.73	98.20 ± 5.19	95.80 ± 2.56

* $P < 0.05$ (two-tailed Student's *t*-test).

drugs or prior treatment of the animals than are the effects upon GAD activities[8]. The apparent parallelism between the fall in glutaminase and GAD, with the drop in GAD being always approximately 15% greater than the drop in glutaminase, is consistent with previous results on rats injected intrastriatally with varying doses of kainic acid and with the suggestion, based on those results, that about 60% of the glutaminase activity in the neostriatum is probably contained in GABAergic neurons.

The apparent protection of cholinergic neurons against the neurotoxic actions of kainic acid is consistent with previous data indicating that taurine may have some membrane stabilizing properties[2,10]. The effect is, however, very slight, suggesting that

kainic acid injections cause an excitation so powerful that it can be affected only slightly by taurine treatment such as is used in these experiments. It is possible that taurine may have a larger therapeutic effect on the relatively slower neostriatal neuropil degeneration found in Huntington's disease, providing that the degeneration is 'excitotoxic' in nature[3,11].

This work was supported by grants from the Medical Research Council of Canada and the Huntington's Chorea Foundation. We thank K. Singh and U. Scherrer for technical assistance and Dr. H. C. Fibiger, Dr. M. Pisa, and Dr. K.-P. Ossenkopp for critical comments.

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PENTOBARBITONE ANAESTHESIA IN AN ANIMAL MODEL OF HUNTINGTON'S DISEASE

to be published in

British Journal of Anaesthesia

Sir - Kainic acid (KA) injections into the rat striatum produce severe loss of striatal neurones, leaving intact both afferent nerve terminals and traversing fibers. The biochemical, morphological and behavioural alterations of KA-injected rats appear to be similar to those found in patients with Huntington's disease (HD) (Coyle *et al*, 1978; Pisa *et al*, 1980; Sanberg *et al*, 1979). Furthermore, the responses elicited in these rats to various pharmacological agents is similar to the responses seen in patients with HD (Mason *et al*, 1978). Therefore, this animal syndrome appears suitable for evaluating the effectiveness of various therapeutic agents for possible use HD patients.

An enhanced sensitivity to anaesthetic drugs in HD patients was reported by Davies (1966) and Gualandi and Bonfanti (1968), but, Farina and Rauscher (1977) were unable to confirm this. Using the KA animal analogue of HD, we were interested in determining how responsive these animals were to pentobarbitone anaesthesia.

Male Wistar rats, weighing about 300g, were stereotaxically injected with 3 nmol of KA in 0.5 µl of phosphate-buffered saline (pH 7.2) into both striata as described elsewhere (Pisa *et al*, 1980; Sanberg *et al*, 1979). Control rats received the injections of phosphate-buffered saline only. Some three months later the rats were injected with either 50 mg/kg or 100 mg/kg of sodium pentobarbitone (Nembutal) i.p. in a volume of 1 ml/kg. Measuring included; a) latency to first motor effect (staggering, falling or no-righting response), b) latency to immobility (no movement for at least 20 sec.), c) latency to disappearance of tail reflex (elicited by gently squeezing a hemostat on the end of the tail every 15 sec.), and d) latency to disappearance of corneal reflex (elicited by gently touching the cornea every 15 sec with a few strands of cotton. Immediately following behavioural testing, the animals were perfused intracardially with isotonic saline solution followed by 10% formol

saline and their brains removed for histological analysis, discussed elsewhere (Pisa *et al*, 1980; Sanberg *et al*, 1979).

The results are shown in Table 1. The KA-lesioned rats showed significantly shorter latencies to first motor effects ($t = 2.66$, $df = 33$, $p < 0.02$) and immobility ($t = 2.96$, $df = 33$, $p < 0.01$), compared to controls, following administration of 50 mg/kg sodium pentobarbitone. However, the KA-lesioned rats did not differ in their latencies to the tail or corneal reflex tests ($ps > 0.05$), compared to controls, at either dose of sodium pentobarbitone. At 100 mg/kg sodium pentobarbitone the KA-lesioned rats showed a significantly shorter latency ($t = 2.29$, $df = 15$, $p < 0.04$) to first motor effects than controls, but did not differ significantly on the immobility test (which was probably due to a ceiling effect).

These results demonstrate that rats with KA-induced striatal lesions show an enhanced sensitivity to the motor inhibiting effects of pentobarbitone, but not to the inhibitory effects on corneal or pain reflexes. These findings thus suggest that HD patients may have enhanced sensitivity to the motor inhibiting effects of sodium pentobarbitone, but not to the anaesthetic effects of this drug. On anaesthetizing patients with these or similar disorders it could be misleading to interpret an abnormally fast induction of motor depression as an index of increased sensitivity to the anaesthetic effects of sodium pentobarbitone.

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Table 1

EFFECT OF KAINIC ACID-INDUCED STRIATAL LESIONS ON LATENCY TO THE MOTOR INHIBITING AND ANAESTHETIC EFFECTS OF SODIUM PENTOBARBITONE[†]

DOSE	FIRST MOTOR INHIBITING EFFECTS[††] (n)	IMMOBILITY (n)	TAIL REFLEX (n)	CORNEAL REFLEX (n)
50 mg/kg				
Control	136.4±7.5 (17)	185.1± 7.7 (17)	340.2±22.0 (9)	423.7±41.9 (9)
KA-lesioned	108.8±7.9 (18)*	144.9±11.8 (18)**	323.3±22.7 (9)	385.6±37.4 (9)
100 mg/kg				
Control	75.8±3.3 (10)	87.5± 2.4 (10)	180.8± 6.6(10)	175.8± 7.1(10)
KA-injected	64.6±3.5 (7)*	88.7± 4.5 (7)	171.7±10.3 (7)	203.7±17.1 (7)

[†]Data are presented as mean seconds±standard error of the mean for n rats in each group.

[††]Included are staggering, falling, and lack of righting responses.

*Significantly different from controls, $p < 0.05$.

**Significantly different from controls, $p < 0.01$.

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Glutamate Neurotoxicity and Tardive Dyskinesia

SIR: We read with interest the letter to the editor "MSG: A Possibility in Treating Tardive Dyskinesia" by Peter J. Holland, M.D. (December 1979 issue). Dr. Holland's suggestion that glutamate may be useful in treating tardive dyskinesia should be regarded with extreme caution.

Microinjection of glutamate or an analogue, kainic acid, into the rat striatum destroys intrinsic neurons, but spares axons of passage and the termination of extrinsic neurons (1). The biochemical, anatomical, and behavioral sequelae appear remarkably similar to those of Huntington's chorea (1, 2). Based on these results and the fact that the striatum receives a major glutamatergic pathway from the neocortex, it has been proposed that excessive glutamate neurotransmission may offer an explanation for the degeneration that occurs in patients with Huntington's chorea (1, 2).

This excitotoxic hypothesis of the pathogenesis of Huntington's chorea also leads to an explanation of tardive dyskinesia, which occurs after long-term therapy with dopaminergic blockers. Treatment with neuroleptics removes the presumed inhibition of the dopaminergic nigro-striatal pathway onto the striatal cholinergic interneurons. In addition, it is possible that neuroleptics may block presynaptic dopaminergic inhibition located on the terminals of the glutamatergic cortico-striatal pathway (3). Thus, the lack of this inhibition could cause an otherwise normal excitatory glutamatergic input from the cortex to become relatively excessive and to gradually destroy striatal interneurons. This would result in a gradual shift in the dopamine-acetylcholine balance in the striatum, a biochemical condition thought to underlie involuntary movements (4). Dr. Holland suggested that treatment with glutamate may normalize this shift by producing an increase in acetylcholine. This appears unlikely because glutamate has been demonstrated to stimulate the release of dopamine from the terminals of the nigro-striatal pathway (5), which would be contraindicated in the treatment of tardive dyskinesia.

As Alan J. Gelenberg, M.D., and associates pointed out in their reply to Dr. Holland (December 1979 issue), there is debate over how much glutamate can penetrate the blood-brain barrier. However, we feel that the increasing number of supportive studies on the neurotoxic properties of glutamate and its analogues further suggests that glutamate may be potentially dangerous in treating tardive dyskinesia and related disorders.

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Dr. Holland Replies

SIR: Drs. Sanberg and Mark raised two important considerations regarding MSG. 1) How much, if any, glutamate crosses the blood-brain barrier? and 2) What potential neurotoxic effects might glutamate have in the CNS?

In a review of recent literature, Kizer and associates (1) remind us that as late as 1951 large doses of MSG (20–30 g/day) were being administered to treat retardation and petit mal, without apparent lasting effect.

The Chinese have used seaweed extract containing glutamate for at least 1,000 years. Today, tons of MSG are added to food merely to enhance flavor. Knowledge of MSG's effects in man is inadequate. The potential uses and hazards of this ubiquitous compound need to be defined.

I thank Drs. Sanberg and Mark for their interest in my hypothesis and hope that this dialogue stimulates the much needed research in this field.

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CONCLUDING REMARKS

With the use of kainic acid to destroy selectively neuronal perikarya in the rat striatum, we were able to show that this part of the basal ganglia is involved in motor, psychological and ingestive behaviours. The interpretation of these results depends on the selectivity of destruction of neuronal cell bodies after kainic acid injections. In our hands, kainic acid produced destruction to striatal neurons, and did not affect traversing or terminating fiber tracts. A number of investigators, however, have demonstrated a loss of afferent terminals in the striatum after kainic acid injections (Butcher and Rogers, 1978; Meibach *et al.*, 1978, Wuerthele *et al.*, 1978). The dose, volume, infusion rate, and stability of the solution (McGeer and McGeer, 1978); the rat strain (Sanberg *et al.*, 1979), and the nature and amount of anaesthetic (Zaczek *et al.*, 1978) can all affect the action of kainic acid. The investigators that have questioned the specificity of kainic acid in the striatum have used from 4 to 15 times the dose, in at least twice the volume, and with faster infusion rates than those utilized in the Thesis. These differences could lead to much more severe destruction in the striatum than demonstrated presently and may account for the discrepant results obtained by various laboratories.

In addition, the multifocality of the kainic acid-induced lesions clearly complicates the anatomical interpretation of the behavioural results. Thus, although the greater severity, symmetry and reliability of occurrence of striatal neuronal loss compared with that of extrastriatal structures might be taken to suggest a main role of striatal damage in the observed

behavioural impairments, a contribution of the extrastriatal pathology cannot be presently excluded.

Although diffusion of kainic acid from the striatal injection site might account for the neuronal loss in close structures such as frontal cortex and globus pallidus, the mechanism of neuronal loss in distant areas such as the hippocampus and the pyriform cortex remains to be fully elucidated. Recent studies, however, have shown that the hippocampal neuronal loss induced by extra-hippocampal injections of kainic acid can be prevented by pre-treatment with antiepileptic drugs, such as diazepam, suggesting that this damage is caused by intense seizure activity (Ben-Ari *et al.*, 1978, 1980; Nadler *et al.*, 1981; Schwob *et al.*, 1980). It is thus possible that the combined use of antiepileptic drugs and kainic acid striatal injections might induce neuronal loss well localised to the striatum, thus preventing ambiguities in the anatomical interpretation of the behavioural sequelae. Indeed in later studies we found this to be the case (Sanberg, 1980; Part 2.2.3).

In birds it may be easier to keep kainic acid induced lesions within the basal ganglia. Studies discussed and presented in the Thesis supported the hypothesis that the avian paleo-striatum is homologous to the mammalian basal ganglia. Injections of kainic acid into the paleostriatum do not produce seizure activity and appear to confine the lesion to the local site of injection (Rieke, 1980). Further studies seem warranted to investigate these differences between birds and mammals.

Unfortunately, kainic acid injections into the paleo-striatum complex destroy the homologues of the striatum and globus pallidus. Therefore, at present it is not possible to dissect the role of the bird 'striatum' in behaviour. Recently,

however, Brown and Grossman (1980) showed that iontophoretic applications of small amounts of kainic acid to the rostromedial zona incerta of rats destroyed this region but not any neighbouring areas in the diencephalon. It is possible that the adaption of this technique to birds may provide a better tool for lesioning selectively separate nuclei within the avian basal ganglia, thus allowing better interpretation of the possible roles of these paleostriatal areas in the behaviour of birds.

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APPENDICES

Life Sciences, Vol. 22, pp. 451-460 Pergamon Press
Printed in the U.S.A.

AMPHETAMINE-INDUCED LOCOMOTOR ACTIVITY AND STEREOTYPY AFTER KAINIC ACID LESIONS OF THE STRIATUM

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Summary

Kainic acid injections were used to destroy cell bodies in the striatum without affecting afferent terminals or fibres of passage. Substantial decreases in choline acetyltransferase and glutamic acid decarboxylase were found particularly in the dorsal half of the striatum but no alteration in the marker enzyme for dopamine terminals, tyrosine hydroxylase. The locomotor activity inducing and stereotypical effects of the psychomotor stimulant drug, d-amphetamine, were tested in these animals and a marked and consistent increase in the effects of amphetamine was found on both measures. This is interpreted in terms of a disruption of the striatonigral feedback system and as suggesting a possible dissociation of function within the striatum between the dorsal and the ventral parts.

Kainic acid, a structural analogue of glutamate (1), when injected into the striatum (caudate-putamen) has been reported to produce neurochemical sequalae similar, if not identical, to those found in post-mortem assay of striatal tissue from human patients suffering from Huntington's disease (2,3). A number of biochemical (4,5,6,7) and behavioural studies (Sanberg, Lehmann and Fibiger submitted) have confirmed the similarity between the symptoms of Huntington's disease and those found in the putative kainic acid animal model.

One of the best documented functions of the striatum is its role in mediating the motor stereotypies (8) seen after administration of high doses of d-amphetamine. This stereotypy-producing effect seems to depend on the integrity of the dopaminergic pathway (9,10) ascending from the substantia nigra to terminate in the striatum (11-17). A related dopaminergic pathway ascending in part from the ventral tegmental area (9,10) and innervating the nucleus accumbens and other mesolimbic and mesocortical areas has been implicated in the mediation of the locomotor hyperactivity produced by lower doses of d-amphetamine (17-21). The major effect of amphetamine and other drugs, such as L-DOPA, which increase the availability of dopamine in the synaptic cleft, (22,23), when administered to patients suffering from Huntington's disease (24,25,26) or the related tardive

diskynesias (27-30), seems to be a marked exacerbation of the choreiform movements, i.e., an increase in motor activity much greater than that produced by the same dose in normal humans. It was thus of interest to determine if the locomotor and stereotypical effects of d-amphetamine were enhanced in the kainic acid animal model, which, if true, would strengthen the correspondence between it and the human disease states.

Materials and Methods

Ten male albino Woodlyn rats weighing about 300 gms were anaesthetised with Nembutal (50 mg/kg intraperitoneal) and positioned in a stereotaxic (Kopf instruments). Two holes were drilled in the skull and 3 nanomoles of kainic acid dissolved in 0.5 microlitre of phosphate buffer, pH 7.2, were injected via a 34 gauge cannula bilaterally over a 5 minute period at the following co-ordinates taken from Konig and Klippel (31), AP + 8.4 mm, ML $\pm$ 2.2 mm, and DV + 0.8 mm. After injection the cannula was left in place for a further 10 minutes to allow diffusion of the drug solution. Ten control rats received injections of phosphate buffer vehicle alone. Two weeks were allowed for recovery after the operation before behavioural testing commenced.

The locomotor response to 1 mg/kg of d-amphetamine was assessed in photocell activity cages measuring 2 ft in diameter, painted black internally and being transected by three infrared photocell beams, interruption of which incremented electromechanical counters located at a distance from the cages. Photocell beam interruptions were cumulated over 10 minute periods and then recorded by an automatic printout counter (BRS instruments). Prior to injection of the drug solution each animal was habituated to the test apparatus for one hour and spontaneous locomotor activity recorded. After this habituation the animal was briefly removed and injected intraperitoneally with the appropriate drug solution and returned to the apparatus where the drug induced locomotor activity was recorded every 10 minutes for two hours. Dextro-amphetamine sulphate (1 mg/kg; Smith, Kline & French) and distilled water vehicle were injected intraperitoneally in two separate sessions being separated by at least two drug free days.

The stereotypy in response to d-amphetamine was measured in the home cage using the stereotypy rating scale of Kelly, Seviour and Iversen (17). A rating of 7 was created for biting and licking of the animal's own body. Stereotypy was rated in the home cage every 10 minutes for two hours in response to the intraperitoneal injection of 10 mg/kg d-amphetamine sulphate dissolved in distilled water.

A two factor repeated measures analysis of variance (32) was used to evaluate the significance of the data.

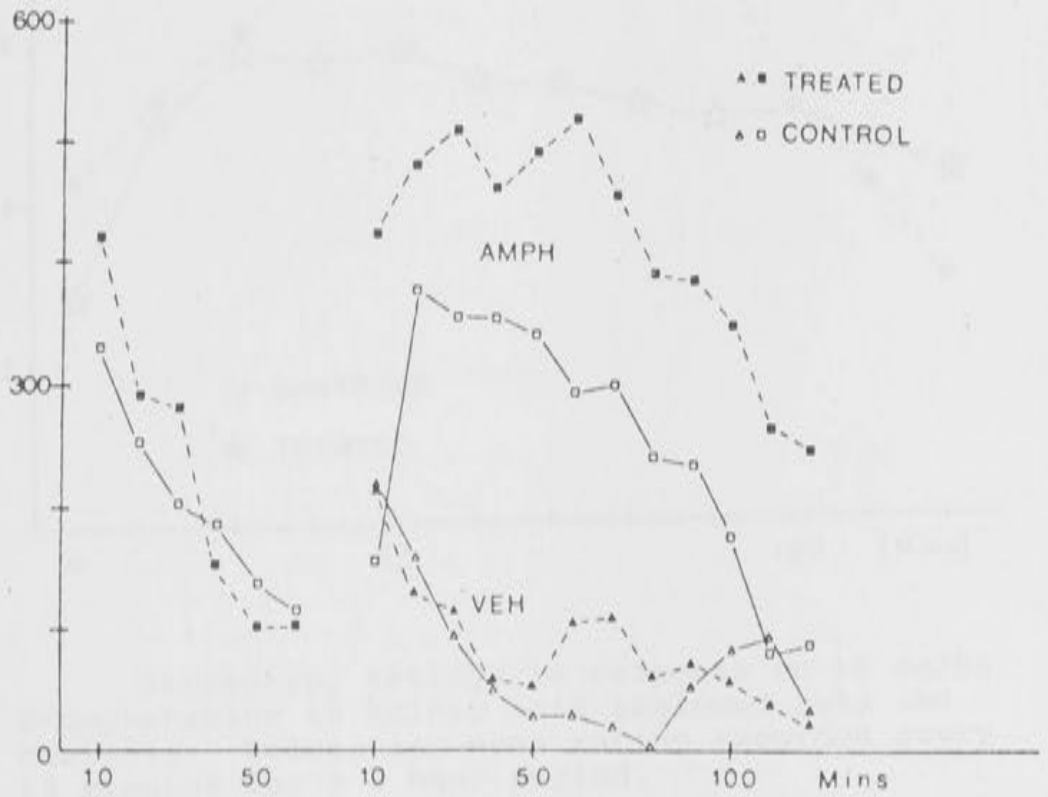
After completion of behavioural testing the animals were allowed at least four drug free days, and then 4 treated and 2 control rats were sacrificed for histological examination of the injection site. Sections were cut from frozen brain tissue at 50 microns and every third section was stained with cresyl violet and counter stained with Luxol blue to reveal both cell

bodies and fibre bundles. A further 3 treated and 3 control rats were sacrificed by cervical fracture and the brain dissected into striatum, accumbens and cortical regions and the activities of the following enzymes assayed; choline acetyltransferase (CAT) by the method of McCaman & Dewhurst (33), glutamic acid decarboxylase (GAD) by the method of Chalmers et al (34) and tyrosine hydroxylase (TOH) by the method of McGeer et al (35). In 2 additional treated and 2 control rats the above regions were dissected out and then the striatum further divided into four quadrants by a vertical and horizontal cut, producing a dorsomedial, a dorsolateral, a ventromedial and ventrolateral portion. These subareas were then assayed for the three enzymes as described above.

Results

The locomotor response to 1 mg/kg of d-amphetamine is shown in Figure 1. As can be seen a marked enhancement of the locomotor stimulant effects of amphetamine was observed in the kainic acid lesioned group, (between groups F ratio = 8.44, df = 1,18, $p < 0.01$). No significant difference was seen either in the spontaneous locomotor activity during the habituation phase or the activity in response to vehicle injections (Figure 1). This indicates that the locomotor activity differences seen after

FIG. 1



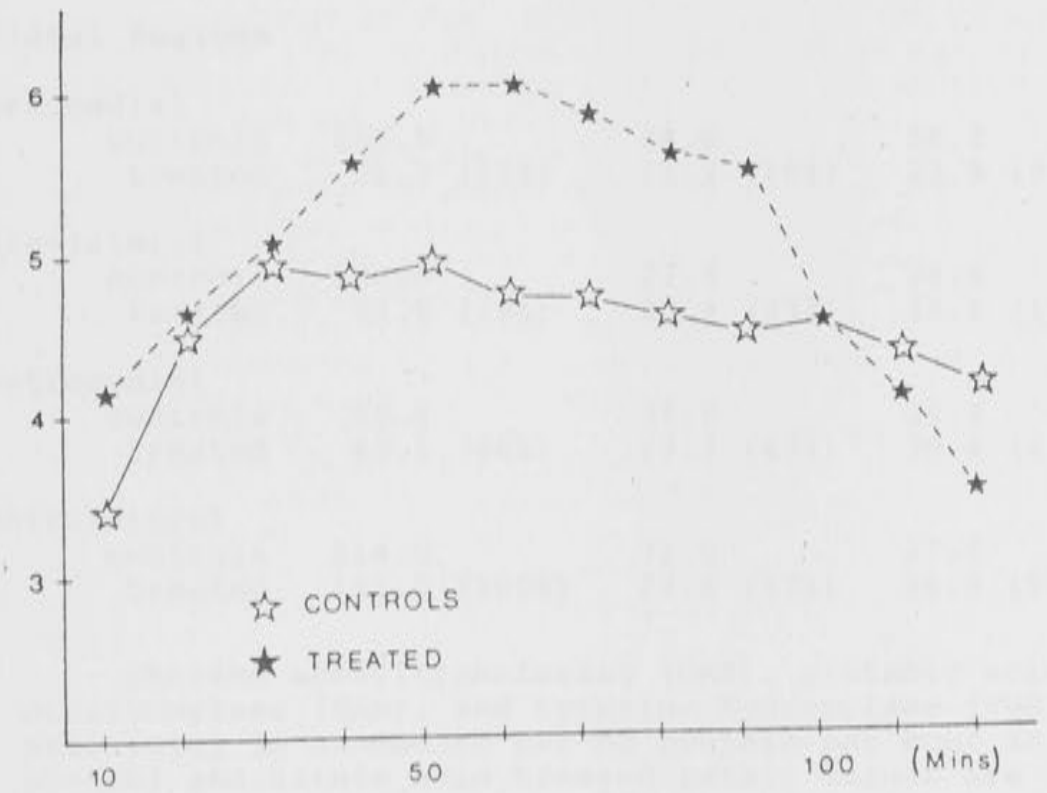
Locomotor activity in response to 1 mg/kg d-amphetamine in kainic acid lesioned rats and controls. Values are mean photocell beam interuptions per 10 minutes for a 1 hour habituation (on left) and for a 2 hour period after injection of drug (AMPH) or vehicle (VEH)(on right).

injection of 1 mg/kg amphetamine were due to the alteration by kainic acid lesion of the response to amphetamine per se and not merely to an alteration in spontaneous baseline activity levels.

The stereotypy response to 10 mg/kg of d-amphetamine was also significantly enhanced in the kainic acid lesioned group, (interaction F ratio = 2.91, df = 11,198, $p < 0.001$) and is shown in Figure 2. Significantly more ratings of the values of 5 or 6, indicative of intense stereotypy (17), were seen in the kainic acid lesioned group than in the controls.

Histological reconstruction of the lesion site showed that although considerable loss of cell bodies had taken place, the fibres of passage of the internal capsule were still intact in the self-same region of the striatum. The lesions appeared to be greatest in the anterior dorsal half of the striatum with sparing of cell bodies in the more ventral parts.

FIG. 2



Stereotypy ratings in response to 10 mg/kg d-amphetamine in kainic acid lesioned rats and controls. Values are mean rating recorded every 10 minutes for a 2 hour period.

The biochemical assay data are shown in Table 1 and it can be seen that whole striatum was depleted by 25% in CAT levels. This modest depletion was in fact shown by regional assay to consist of a 55% depletion in CAT and GAD levels in the dorsomedial quadrant, significant depletion in the dorsolateral portion (CAT 32%: GAD 63%) but very little effect in the ventrolateral or ventromedial parts. Thus, the biochemistry concurs

TABLE 1

Enzyme Activities In Kainic Acid Lesioned Brain Areas

Area	CAT	GAD	TOH
Cortex			
controls	23.3	-	-
treated	20.6 (88%)	-	-
Accumbens			
controls	63.7	62.6	13.6
treated	58.6 (92%)	60.3 (97%)	15.4 (113%)
Whole Striatum			
controls	52.6	-	12.2
treated	40.1 (76%)	-	14.1 (115%)
Striatal Regions			
Dorsomedial			
controls	108.0	24.8	26.2
treated	51.2 (47%)	11.2 (45%)	23.9 (91%)
Dorsolateral			
controls	138.0	27.9	29.6
treated	94.8 (68%)	10.4 (37%)	34.6 (117%)
Ventromedial			
controls	80.8	37.0	26.9
treated	69.1 (86%)	23.3 (63%)	30.4 (113%)
Ventrolateral			
controls	114.0	32.0	27.6
treated	115.0 (100%)	27.8 (87%)	26.9 (98%)

Choline acetyltransferase (CAT), glutamic acid decarboxylase (GAD), and tyrosine hydroxylase (TOH) activities in nanomoles per mg protein per hour in control and kainic acid treated rats. Values are means of 5 rats for cortex and accumbens, 3 rats for whole striatum and 2 rats for regional striatum as stated in text. Figures in parentheses are percentage of control activity remaining in kainic acid lesioned tissues.

with the histology in suggesting that the lesion was mainly localized to the dorsal part of the striatum. Both histology and biochemistry (Table 1) showed that no damage occured in either the nucleus accumbens or in the cortex overlying the injection site. Tyrosine hydroxylase was not affected, even in the regional assays, suggesting that the dopamine terminals were not directly affected.

Discussion

The enhancement of both the locomotor and stereotypy inducting actions of d-amphetamine after kainic acid lesions of the striatum clearly increases the similarities of this animal model with human disease states such as Huntington's chorea (2,3). This enhancement has been found at other doses of amphetamine for both locomotor and stereotypical effects (Mason & Fibiger, in preparation) and so is a general phenomenon of a shift in the dose-response curve of d-amphetamine to the left. The present data thus add to the previously reported biochemical (4,5, 6) and behavioural (Sanberg, Lehmann & Fibiger, submitted) similarities between the kainic acid animal model and Huntington's disease in human patients.

It is, however, paradoxical that severe destruction of cell bodies in the striatum leads to an increase, rather than a decrease, in the amphetamine response. Large electrolytic lesions which destroy the entire striatum unambiguously block the stereotypy response to amphetamine (36,37), as do 6-hydroxydopamine (6-OHDA) lesions which deplete dopamine in the whole striatum (13,38). The striatum is not, however,a homogenous structure since electrolytic (39) or 6-OHDA (40) lesions of the ventral portion are effective in blocking amphetamine stereotypy but 6-OHDA lesion to the dorsal striatum does not (40). Moreover, electrolytic lesion in the dorsal striatum leads to an enhancement of the amphetamine response (39). A similar dissociation between the dorsal and ventral striatum is indicated in the control of active avoidance performance (41), consummatory responses (42), and intracranial self-stimulation performance (Neill, Peay and Gold, submitted). Thus, our lesions which were successful in severely damaging cell bodies in the dorsal anterior striatum but sparing much of the ventral part of this structure, as shown by histology and regional biochemical assay, correspond more closely to the dorsal electrolytic lesions of Neill, Boggan and Grossman (39) which did indeed lead to an increase in the amphetamine response. This suggests that the motor outflow from the striatum necessary for the expression of stereotypy and damaged by global striatal lesions may be more localized to the ventral half (40).

It is still necessary, however, to explain why dorsal striatal lesions increase the amphetamine response, rather than having no effect as a ventrally located motor outflow model on its own would suggest. Bunney & Aghajanian (personal communication) have found that kainic acid injection into the striatum which destroyed striatal cells but had no effect on the more ventral nucleus accumbens, abolished the usual inhibition of single units in the substantia nigra (43,44) in response to intravenous d-amphetamine. This suggests that part of the striato-nigral feedback loop (45,46) may have been destroyed by the kainic acid lesion, and that the effects of amphetamine may be two-fold; (1) to release dopamine in the accumbens to produce locomotor activity (17-21) or in the ventral striatum to produce stereotypy (8,37,40,41,42), and (2) to activate a striato-nigral negative feedback loop which inhibits the activity of dopaminergic neurons in the substantia nigra, pars compacta

(SNC). Since it is known that the amount of dopamine released onto the post-synaptic cell is a combined function of amphetamine and the firing rate of SNC neurons (23), the amount of dopamine being released onto the post-synaptic receptors in striatum or accumbens would be a balance of these two, opposing actions of amphetamine. Therefore, if a substantial portion of striatonigral feedback loop originates in the dorsal striatum, while the primary motor output region is located in the nucleus accumbens and ventral striatum, then a selective lesion of the dorsal striatum would result in enhanced release of dopamine in accumbens and ventral striatum and hence increase the motor effects of amphetamine. This model assumes that the mesolimbic projection to the accumbens is also under feedback control from the striatum. In this regard, it is of interest that it has recently been shown that accumbens dopamine projections do indeed originate in part from medial A9 (47) cells, not exclusively from the A10 area (48,49).

Thus, it is tentatively proposed that a disruption of feedback inhibition may explain the observed increase in the amphetamine response after kainic acid lesion of the dorsal striatum reported here.

Acknowledgements

We thank J.I. Nagy, J. Lehmann and F. LePiane for assistance. STM is an MRC Fellow. Supported by the MRC.

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Kainic Acid Lesions of the Striatum Dissociate Amphetamine and Apomorphine Stereotypy: Similarities to Huntington's Chorea

Abstract. *Kainic acid lesion of cell bodies in the dorsal striatum enhanced the stereotypy-producing effects of* d*-amphetamine without affecting the stereotypy produced by the direct receptor agonist apomorphine. This pattern of results parallels that found in patients suffering from Huntington's chorea, thus strengthening the parallels between the kainic acid animal model and the human disease state initially suggested on biochemical grounds. The present results further suggest a dissociation of the mechanisms involved in the production of stereotypy by these two drugs, perhaps in terms of differential involvement of the striato-nigral negative feedback loop.*

Huntington's chorea is a genetically transmitted autosomal dominant degenerative disease (*1*) characterized by involuntary choreic movements (*2*). Evidence indicates an involvement of the basal ganglia in this disease (*3*), and it has been proposed that rats in which kainic acid has been injected into the striatum provide a model (*4*) that shows considerable biochemical similarities (*5*) to the human disease state. One of the best demonstrated functions of the striatum in animal experiments has been a role in the mediation of the stereotyped behavior patterns (*6*) seen in animals given high doses of the psychomotor stimulant drug amphetamine (*7*, *8*). Electrolytic lesion of the entire stratum convincingly blocks amphetamine stereotypy (*9*), as does total destruction of afferent dopamine terminals by means of the selective neurotoxin 6-hydroxydopamine (6-OHDA) (*10*). On the other hand, amphetamine and other dopamine releasing agents, when administered to patients with Huntington's chorea, seem to exacerbate the choreic movements (*11*), that is, they have an enhanced effect compared to an identical dose in normal humans. L-Dopa has, in fact, been suggested to be of use in revealing "presymptomatic" chorea in otherwise apparently normal humans (*12*). Apomorphine, a direct dopamine receptor agonist (*13*), is devoid of this effect (*14*) despite its being highly effective in elicit-

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ing stereotyped behaviors in the rat (*15*).

It would considerably strengthen the parallel with the human disease state if this dissociation of the action of amphetamine and apomorphine could be demonstrated in the kainic acid animal model (*16*). We therefore injected kainic acid, a structural analog of glutamate (*17*), into the striatum of the rat, and examined the stereotypy elicited by various doses of amphetamine and apomorphine. Kainic acid has been shown to damage cell bodies in the striatum while sparing afferent terminals and fibers of passage such as those of the internal capsule (*4*, *5*).

Ten male, albino Woodlyn rats, each weighing about 300 g, received bilateral injections of 3 nmole of kainic acid in the striatum (*18*). Ten control rats were injected with the vehicle. The stereotypy induced in these animals by intraperitoneal injections of *d*-amphetamine (5 and 10 mg/kg) and by apomorphine (1, 2, and 4 mg/kg) was rated according to the scale of Kelly *et al.* (*8*, *19*). Stereotypy was rated in the home cage every 10 minutes for 2 hours following the intraperitoneal injection of *d*-amphetamine and for 1 hour after injection of apomorphine. Drugs were dissolved in distilled water with, in the case of apomorphine, ascorbic acid (0.3 mg/ml) added to retard oxidation. The mean stereotypy scores in response to amphetamine and apomorphine are shown in Fig. 1. The rats with kainic acid lesions showed a marked and consistent increase in stereotypy in response to amphetamine; however, no alteration occurred in the stereotypy induced by apomorphine (*20*). Both doses of amphetamine produced an enhanced response, whereas none of the three doses of apomorphine produced responses that were significantly affected by the kainic acid lesion (*21*).

Although the peak intensity of the amphetamine stereotypy was increased, its speed of onset was apparently unaltered (Fig. 2). This might be taken to suggest that a negative feedback mechanism, which comes into play to limit the intensity of stereotyped behavior in its most extreme and intense forms, is missing in the animals with kainic acid lesions.

After completion of the behavioral testing, four treated and two control rats were killed and their lesion sites were examined histologically. Frozen sections of brain were cut at 50 μm, and every third section was stained with cresyl violet and counterstained with Luxol blue to reveal cell bodies and fiber bundles, respectively. A typical section through the site of kainic acid injection (Fig. 2) shows considerable damage occurring to cell bodies in the dorsal striatum but significant sparing of the more ventral parts; fiber bundles appear to be completely spared, even in the regions of maximum cell body loss. No damage could be observed in either the adjacent nucleus accumbens or in the cortex overlying the injection site.

A further three treated and three control rats were used for biochemical analysis. The brain of each rat was dissected into striatum, accumbens, and cortical regions and then the striatum was further subdivided by a horizontal and a vertical cut into four quadrants, thus giving a

Table 1. Choline acetyltransferase (CAT), glutamic acid decarboxylase (GAD), and tyrosine hydroxylase (TOH) activities in control and kainic acid–treated rats. The data are expressed in nanomoles per milligram of protein per hour. Values are means of three control and three treated animals (± standard error of the mean).

Area and group	CAT		GAD		TOH	
	Activity	Percentage*	Activity	Percentage*	Activity	Percentage*
Cortex						
Control	23.3 ± 0.57					
Treated	20.6 ± 1.71	88				
Accumbens						
Control	63.7 ± 2.32		62.6 ± 5.95		13.6 ± 0.56	
Treated	58.6 ± 1.54	92	60.3 ± 4.8	97	15.4 ± 0.94	113
Striatal regions						
Dorsomedial						
Control	108.0 ± 5.4		24.8 ± 0.3		26.2 ± 1.0	
Treated	51.3 ± 6.15	47	11.3 ± 2.26	45	23.9 ± 0.15	91
Dorsolateral						
Control	138.6 ± 13.5		27.9 ± 1.65		29.6 ± 0.85	
Treated	94.8 ± 19.5	68	10.4 ± 1.28	37	34.7 ± 0.75	117
Ventromedial						
Control	80.8 ± 1.45		37.0 ± 5.85		26.9 ± 0.75	
Treated	69.1 ± 6.3	86	23.3 ± 6.1	63	30.4 ± 1.35	113
Ventrolateral						
Control	114.5 ± 10.05		32.0 ± 4.5		27.6 ± 1.6	
Treated	115.3 ± 10.85	100	28.0 ± 3.35	88	27.0 ± 2.75	98

*Percentage of control activity remaining in kainic acid–lesioned tissue.

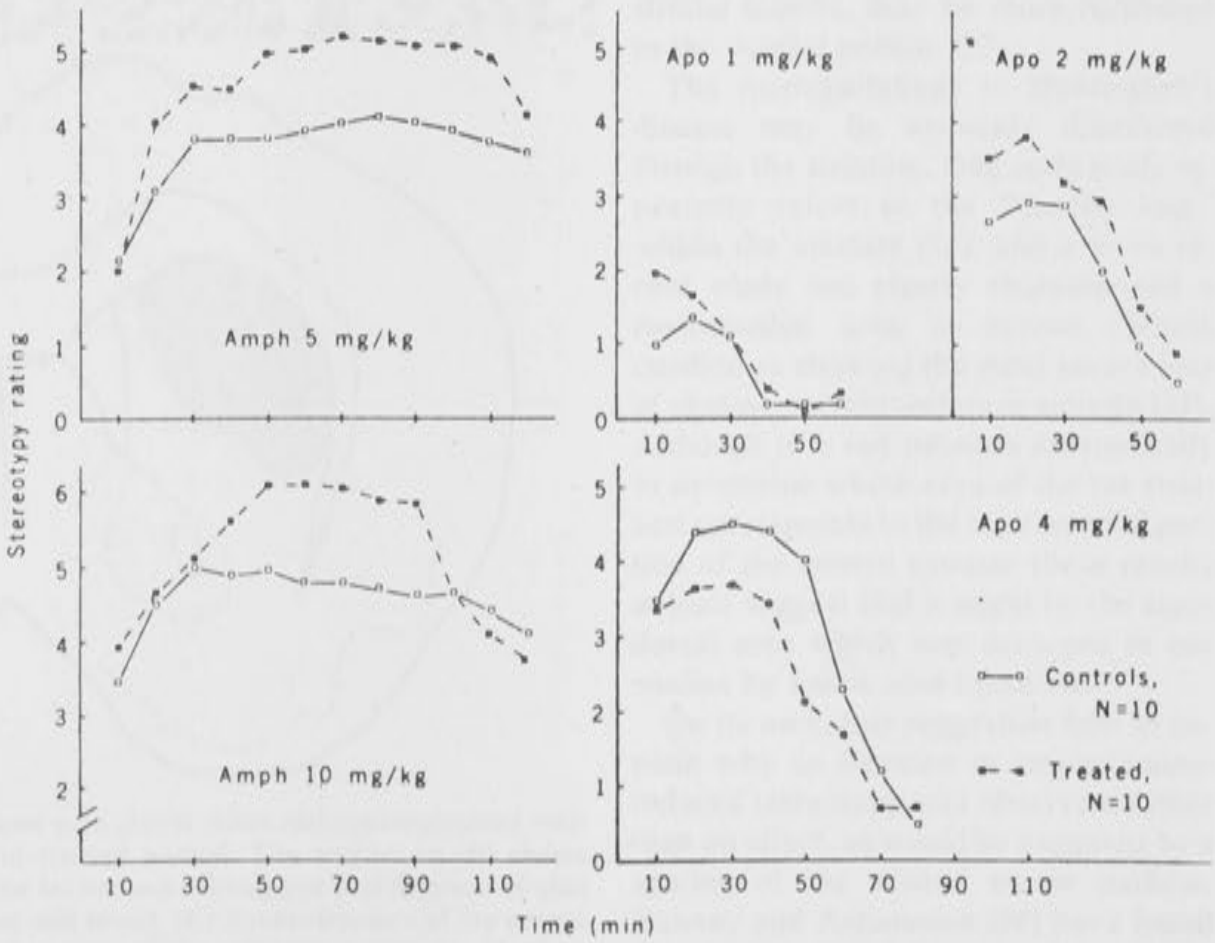


Fig. 1. Stereotypy response to amphetamine (5 and 10 mg/kg) and apomorphine (1, 2, and 4 mg/kg) in control and kainic acid–treated animals. Values are means for ten control and ten treated rats. The stereotypy score, according to the rating scale of Kelly *et al.* (*8*), is plotted against time for 2 hours after the injection of *d*-amphetamine and for 1 hour after the injection of apomorphine.

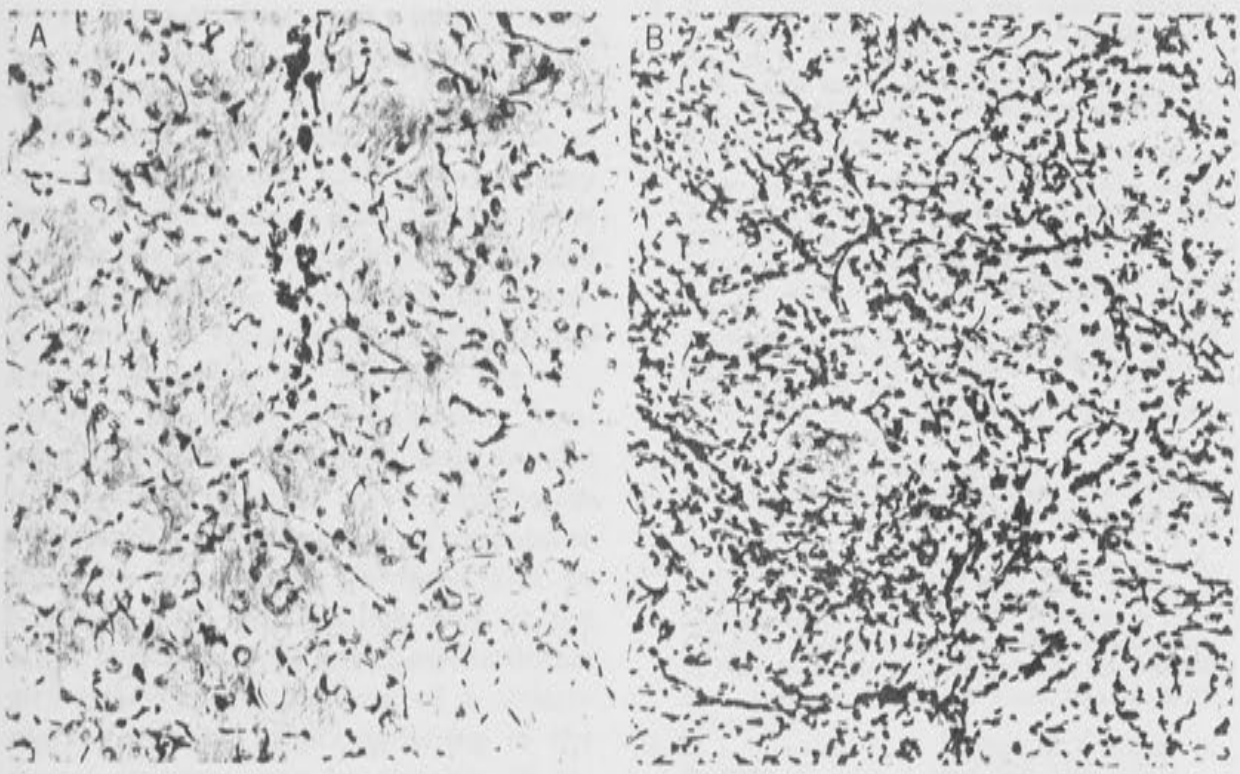
dorsomedial, dorsolateral, ventromedial, and ventrolateral subarea. These regions were assayed for the activity of the enzymes choline acetyltransferase (E.C. 2.3.1.6) (*22*), glutamate decarboxylase (E.C. 4.1.1.15) (*23*), and tyrosine hydroxylase (E.C. 1.14.16.2) (*24*). The biochemical data (Table 1) agree with the histological examination in showing that the lesion was mainly confined to the dorsal aspect of the striatum with considerable sparing of enzyme activities in the ventral portion and with no alteration in cortex or accumbens. The specificity of kainic acid for cell perikarya is shown by the marked decrease in the dorsal portion of the striatum in those enzymes associated with cells whose soma are intrinsic to the striatum (choline acetyltransferase and glutamate decarboxylase) but with no alteration in the marker enzyme (tyrosine hydroxylase) for the afferent dopamine terminals coming from areas outside the striatum.

The finding that the stereotypy response to amphetamine is increased in rats with kainic acid lesions of the striatum thus parallels that found in Huntington's disease, especially since the apomorphine response is unaltered in both instances. This adds to the previously described biochemical similarities (*5*) between the kainic acid animal model and the human disease state. The consistent increase in the amphetamine response makes the lack of effect on the apomorphine response in the selfsame animals all the more marked.

It is, at first sight, paradoxical that destruction of cell bodies in the striatum should increase amphetamine-induced stereotypy, because destruction of the striatum in other ways usually blocks this stereotypy (*9*, *15*), and lesions made with 6-OHDA, which deplete dopamine in the whole striatum, also attenuate (*25*) or completely block it (*7*–*9*). The striatum is not a homogenous structure, since smaller lesions (restricted to the ventral part) made electrolytically (*26*) or with 6-OHDA (*27*) are equally effective in blocking amphetamine-induced stereotypy, but lesions made with 6-OHDA in the dorsal striatum (*27*) are without effect and electrolytic lesions of that area increase the amphetamine response (*26*). Similar dissociation of dorsal and ventral striatum is found in the control of active avoidance (*28*), consummatory behavior (*29*), DRL (differential reinforcement of low rate) performance (*30*), and modulation of intracranial self-stimulation (*31*). Thus, our kainic acid lesion, which was restricted to the dorsal aspect of the striatum, is more similar to the electrolytic lesion of Neill *et al.* (*26*), both in location and in terms of increasing the amphetamine response. This suggests that the motor outflow from the striatum that is necessary for the expression of stereotypy, and is damaged by global striatal lesions, may be more restricted to the ventral portion (*27*).

The neuropathology in Huntington's disease may be unevenly distributed through the striatum. One early study repeatedly refers to the "patchy loss" within the caudate (*32*), and a more recent study has clearly characterized a rostromedial area in human choreic caudate as showing the most severe loss of choline acetyltransferase activity (*33*). Although it is not possible anatomically to determine which area of the rat striatum corresponds to the rostromedial portion of the human caudate these results at least suggest that it might be the same dorsal area which was damaged in our studies by kainic acid injections.

On its own, this suggestion fails to explain why an increase in amphetamine-induced stereotypy was observed, rather than no effect, as would be expected by a sparing of the ventral motor outflow. Bunney and Aghajanian (*34*) have found that injections of kainic acid into the striatum, which destroyed cells in this structure and had effect on the more ventral nucleus accumbens, abolished the



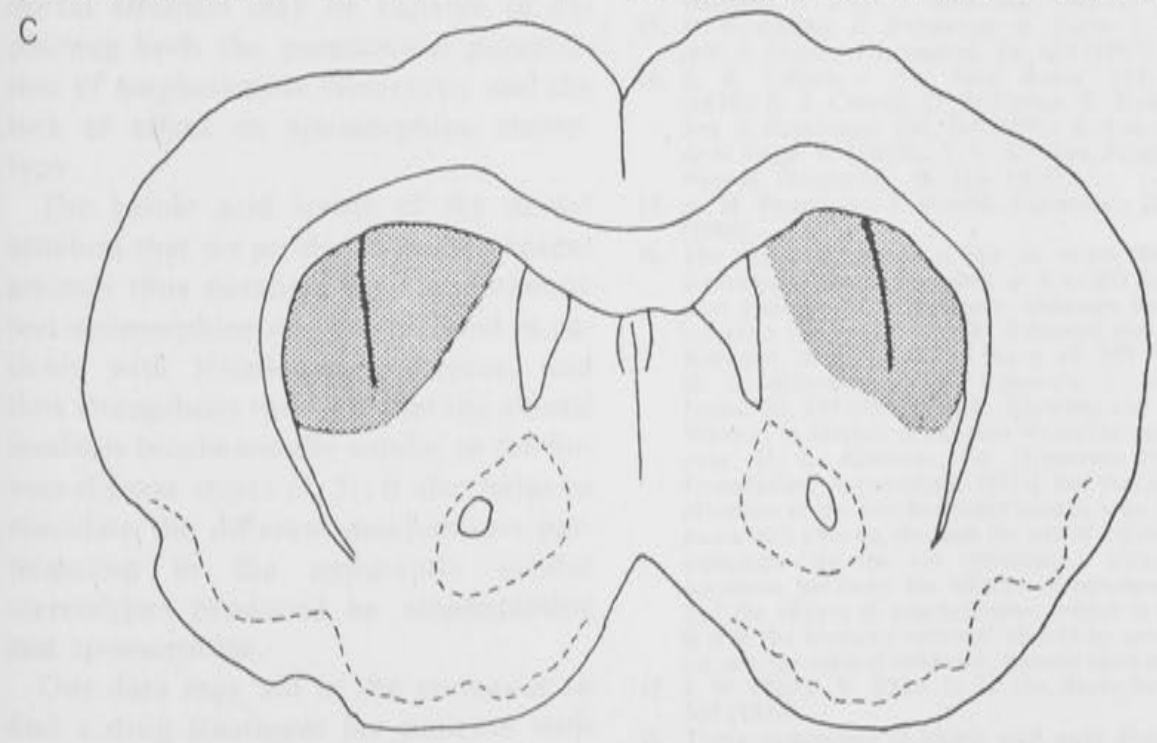


Fig. 2. Section through striatal injection site, stained with cresyl violet and counterstained with Luxol blue. (A) Control animal. (B) Kainic acid–treated animal. The section in (B) shows the marked loss of cell perikarya produced by the lesion and subsequent proliferation of glial cells. In this same area, however, fiber bundles are still intact. (C) Reconstruction of the extent of the lesion. Considerable sparing of the ventral portion of the striatum is evident. Nucleus accumbens and cortex overlying the injection site showed complete sparing of cell bodies, indicating that the lesion did not impinge on these areas. The rostro-caudal extent of the lesion was from approximately A 9650 to A 6570 on the atlas of Konig and Klippel (*18*). No invasion of the globus pallidus was found.

usual inhibition (*35*) of single units in the substantia nigra, pars compacta (SNC), in response to intravenously administered amphetamine. They suggest that the striato-nigral feedback loop (*36–38*) may have been interrupted by the kainic acid lesion. This implies that the effects of amphetamine may be twofold, in that it releases dopamine from the terminals of dopamine neurons in the ventral striatum to cause stereotyped behavior, and inhibits the firing of SNC cells by an action on the striato-nigral feedback loop in the dorsal striatum. Since the amount of dopamine released onto the postsynaptic receptor is known (*39*) to be a combined function of the dose of amphetamine and the electrical activity in SNC neurons, the activation of the postsynaptic receptor in the ventral striatum would be a balance of these two, opposing actions of amphetamine.

Removal of that action of amphetamine which inhibits SNC cells, as may happen after kainic acid lesions have been made in the dorsal striatum, would thus enhance the overall action of amphetamine in releasing dopamine in the ventral striatum to produce the expression of stereotyped behavior. Apomorphine, since it acts directly on the postsynaptic receptor (*13*) would be independent of the release of dopamine from the presynaptic terminal and so would not be affected one way or the other by kainic acid lesion of the dorsal striatum. Thus, disruption of a striato-nigral feedback loop originating in the dorsal striatum may be capable of explaining both the paradoxical potentiation of amphetamine stereotypy and the lack of effect on apomorphine stereotypy.

The kainic acid lesion of the dorsal striatum that we produced in the present animals thus parallels the amphetamine and apomorphine responses found in patients with Huntington's disease, and thus strengthens the view that the animal model is biochemically similar to the human disease states (*4*, *5*); it also helps to elucidate the different mechanisms participating in the apparently similar stereotypes produced by amphetamine and apomorphine.

Our data may aid in the endeavor to find a drug treatment for patients with Huntington's disease, in similar vein to the use of L-dopa for Parkinson's disease. Some progress has been made in examining drugs that may restore the biochemical deficiencies of rats with kainic acid lesions (*40*). There is, however, no guarantee that partial restoration of biochemical function will be adequate to restore behavioral normality, and it is practically difficult, as well as of a doubtful ethical nature, to conduct tests on human patients on the basis of such scanty evidence.

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16. The important point is not so much that rat stereotypy should be taken as a model for human choreiform movements, although this has certainly been suggested [R. Rubovits and H. L. Klawans, *Arch. Gen. Psychiatry* **27**, 502 (1972); H. L. Klawens and R. Rubovits, *J. Neural Trans.* **33**, 235 (1972); H. L. Klawans and W. J. Weiner, in *Models of Human Neurological Disease*, H. L. Klawans, Ed. (Excerpta Medica Foundation, Amsterdam, 1975)], but that by duplication of the biochemical changes seen in humans with chorea, through the use of kainic acid injection into the rat striatum, a clear dissociation between the effects of apomorphine and the effects of amphetamine, which is manifest in the human condition, should be seen in a rat species-typical behavior, namely stereotypy.
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18. Three nanomoles of kainic acid were dissolved in 0.5 μl of phosphate buffer, *p*H 7.2, and injected bilaterally via a 34-gauge cannula over a 3-minute period at the following coordinates [from J. F. Konig and R. A. Klippel, *The Rat Brain, A Stereotaxic Atlas* (Williams & Wilkins, Baltimore, 1963)]: AP + 8.8 mm, ML + 2.8 mm, DV + 0.4 mm. After injection the cannula was left in place for a further 5 minutes to allow diffusion of the drug solution. Two weeks were allowed for recovery after the operation before behavioral testing commenced. After a transient hypophagia during the first week after the operation, body weight recovered to control values by the time of initiation of drug testing. Animals were hyperreactive to handling but otherwise appeared similar to controls and remained healthy throughout the duration of testing.
19. A rating of 7 was created to describe stereotyped licking or biting of the animal's own body.
20. A two-factor analysis of variance [B. J. Winer, *Statistical Principles in Experimental Design* (McGraw-Hill, New York, 1962)] with repeated measures on one factor (time) was carried out for each dose of each drug. The *F* ratios were as follows: amphetamine, 10 mg/kg, interaction F (11, 198) = 2.91, $P < .001$; amphetamine, 5 mg/kg, groups F (1, 18) = 4.96, $P < .05$; apomorphine, 1 mg/kg, groups F (1, 18) = 1.76, N.S.: interaction F (5, 90) = 1.69 N.S.; apomorphine, 2 mg/kg, groups F (1, 18) = 1.95, N.S.: interaction F (5, 90) = 0.76 N.S.; apomorphine 4 mg/kg, groups F (1, 18) = 2.08, N.S.: interaction F (7, 176) = 1.75 N.S.
21. Apomorphine and amphetamine were tested on the same animals with the drug order being amphetamine 5, apomorphine 1, apomorphine 2, apomorphine 4, and amphetamine 10 mg/kg. Four drug-free days were allowed between drug administrations.
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38. Some authors have disputed the existence of a striato-nigral feedback loop [P. M. Groves, C. J. Wilson, S. J. Young, G. V. Rebec, *Science* **190**, 522 (1975)] and the feedback inhibition of amphetamine may actually be mediated by release of dopamine within the substantia nigra itself onto dopamine receptors on the terminals of the descending, possibly γ-aminobutyric acid–containing, fibers from the striatum [K. Gale, A. Guidotti, E. Costa, *Science* **195**, 503 (1977); P. F. Spano, M. Trabucchi, G. Di Chiara, *ibid.* **196**, 1343 (1977); C. E. Riback, J. E. Vangh, K. Saits, R. Barber, E. Roberts, *Brain Res.* **116**, 287 (1976)]. These fibers are known to be destroyed after kainic acid lesion of the striatum [E. G. McGeer and P. L. McGeer, *Nature (London)* **263**, 517 (1976)] and so on either mechanism the feedback action would be blocked by such a lesion. Even Groves and co-workers [G. V. Rebec and P. M. Groves, *Neuropharmacology* **14**, 275 (1975)] report that hemitransection of descending fibers from the striatum blocks the usual inhibition of SNC cells to intravenous amphetamine. The location of this effect is somewhat irrelevant to the present findings. The important point is that both Groves and co-workers and Aghajanian and Bunney (*37*) agree that it occurs.
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12 December 1977; revised 5 April 1978

Reprinted from:
Australian Psychologist 15, 1980, 262-263

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Effects of Lithium Carbonate on Haloperidol-Induced Catalepsy in Rats

Lithium (Li) is commonly used in the treatment of mania. It is frequently used in conjunction with haloperidol in controlling acute manic episodes, or in the treatment of schizo-affectives. Li-haloperidol antagonism has been reported in the clinical literature, at present the mechanism of this antagonism is poorly understood. Studies of neuroleptic-induced changes in dopamine (DA) receptor site binding affinities point towards an explanation in terms of the sensitivity of the DA receptors. Withdrawal from chronic haloperidol administration results in an increase of DA receptor sensitivity, but long term Li administration concurrent with haloperidol prevents this increase in DA receptor sensitivity. However, it has been shown that administration of Li results in a potentiation of acute haloperidol-induced behavioural effects. Acute Li administration may be causing a paradoxical short term increase in the sensitivity of DA receptors to haloperidol. In the following study, experimental rats and their frontal and parietal cortices removed, one effect of which is to permanently remove haloperidol binding to presynaptic DA receptors on the terminals of the cortico-striatal tract. After four weeks recovery, experimental and control animals were randomly allocated to one of four treatment groups: Li and haloperidol (LH), Li alone (L), haloperidol alone (H), and saline (S). All animals were appropriately injected with buffered lithium carbonate (150 mg/kg), and haloperidol (0.5 mg/kg), in isotonic saline vehicles. The animals were tested on a standard catalepsy task, and their descent latencies measured. In the experimental groups LH animals did not differ from H animals on the catalepsy test, although both took significantly longer to descend than L or S animals. LH controls took significantly longer than all other control groups who did not differ in descent latencies. However, the longer latencies for LH controls were significantly shorter than LH experimental animals. This experiment demonstrates that acute administration of Li results in a potentiation of haloperidol-induced catalepsy in normal rats. This Li-induced potentiation does not occur in rats without presynaptic DA receptors in the striatum. Although post-synaptic DA receptors localized on striatal interneurons have been shown to mediate haloperidol-induced catalepsy, it is evident from the present results that pre-synaptic DA receptors on striatal afferents from the cortex may play a role in the potentiation of haloperidol-induced catalepsy by acute Li administration.

Table 1

Descent latencies in normal and decorticate rats following acute injections of either isotonic saline alone, lithium carbonate alone (150 mg/kg), haloperidol alone (0.5 mg/kg), or combined lithium carbonate and haloperidol.[1]

GROUP	NORMAL (N)	DECORTICATE (N)
Lithium Carbonate + Haloperidol	126.58 ± 31.60 (10)	89.33 ± 21.72 (11)
Haloperidol	61.77 ± 32.28 (10)	98.33 ± 25.33 (11)
Lithium carbonate	8.69 ± 2.20 (10)	10.53 ± 1.68 (11)
Saline	8.65 ± 3.44 (10)	12.41 ± 2.87 (11)

[1] The data represent the pooled mean ± standard error in seconds, of two catalepsy tests on each animal.

Physiology & Behavior, Vol. 24, pp. 11–19. Pergamon Press and Brain Research Publ., 1980. Printed in the U.S.A.

Locomotor Activity, Exploration and Spatial Alternation Learning in Rats with Striatal Injections of Kainic Acid

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Received 15 March 1979

PISA, M., P. R. SANBERG AND H. C. FIBIGER. *Locomotor activity, exploration and spatial alternation learning in rats with striatal injections of kainic acid.* PHYSIOL. BEHAV. 24(1) 11–19, 1980.—Bilateral injections of three nmoles of kainic acid into the rat striatum induced a set of behavioral alterations including temporary aphagia and adipsia, abnormal gait, acute and chronically recurrent seizures, interference with onset of ambulatory activity and impairments in performance of instrumental spatial alternation. In addition to a severe loss of striatal neurons in all kainate-treated rats, the kainate injections often resulted in extrastriatal neuronal loss, most consistently involving the pyramidal neurons of the hippocampus. Similar regulatory, locomotor, and learning alterations were however found both in kainate-treated rats with combined striatal and hippocampal lesions and in those with no detectable hippocampal damage, suggesting that the striatal degeneration accounted in large part for the behavioral impairments. Although striatal injections of kainic acid may fail to produce selective neuronal degeneration in the striatum, they result in behavioral disorders that appear to be similar, at least superficially, to those of patients with Huntington's disease, thus encouraging further use of this neurotoxin as a tool for reproducing some aspects of this disease.

Kainic acid Striatal lesions Feeding Drinking Stereotypy Seizures Locomotion
Exploration Alternation d-Amphetamine

INJECTIONS of kainic acid (KA) into the rat striatum cause marked glial proliferation and severe loss of striatal neurones leaving largely intact both afferent nerve endings and fibers of passage [5, 6, 9, 15]. The biochemical and morphological alterations of kainate-injected striata appear to be similar to those found in the caudate-putamen of patients with Huntington's disease (HD) [5, 7, 26, 46, 47].

In HD neuronal degeneration most consistently and markedly occurs in the caudate-putamen and the frontorolandic cortex [22,41]. Various degrees of neuronal loss and gliosis have however been reported in several other forebrain areas such as globus pallidus, hypothalamus, thalamus and hippocampus [2, 3, 12, 22, 25, 29]. Among the main symptoms of HD are included alterations of body weight regulation, choreic movements, abnormal fluctuations of mood, increased distractibility, impairments in spontaneous initiation of activity, and impairments in organization, planning, and sequential arrangement of information [2, 4, 28]. In spite of the traditional wisdom attributing the motor disorders to caudate-putamen pathology and the affective and cognitive disorders to cortical pathology, there is in fact no evidence on the specific anatomical correlates of the behavioral disorders of HD patients.

Several animal studies have reported regulatory, motor, and cognitive impairments in rats with coagulative lesions of the striatum [13, 18, 31, 33, 35, 50, 54]. However, these conventional methods of lesions interrupt cortical projections traversing the striatum, thus complicating the interpretation of the behavioral results. It has therefore been suggested that behavioral studies using animals with KA-induced striatal lesions might afford more selective information on the role of caudate-putamen degeneration in the behavioral disorders of HD patients [9, 24, 43, 44].

In previous studies in this laboratory [24,44] no appreciable alterations could be revealed in the diurnal locomotor activity of rats with striatal injections of KA (KAL rats) measured in photocell cages, although their locomotor response to d-amphetamine was abnormally increased. Also KAL rats showed no impairments in continuously reinforced bar pressing, although they were impaired both in extinction of this response and in suppression of punished step-down responding [44].

The present study examined whether KAL rats have altered locomotor activity under different conditions from those used previously [24,44]. Also, KAL rats were trained on food-reinforced spatial alternation, a task presumably involving sequential structuring of behavior.

Although no damage to extrastriatal structures was de-

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scribed in early investigations on the neuropathologic sequelae of striatal injections of KA [8], the possibility of such damage has been reported more recently, at least after injections of relatively high doses of KA [6,55]. In an early investigation [44] we observed moderate neuronal loss in the neocortex and the hippocampus of a small sample of KAL rats in which the effects of the KA injections were assessed with histological rather than biochemical methods. In the present study, histological analysis was conducted on the brains of all participating KAL rats, to document more extensively the possible occurrence of extrastriatal neuronal loss.

METHOD

Subjects

Twenty male Wistar rats (Woodlyn Farms, Guelph, Ontario) weighing 275–300 g were used. They were housed in groups of six before surgery and in individual stainless steel cages afterwards, with free access to water and Purina Rat Chow food. The colony room had a temperature of 22–25°C, humidity of 45–55%, and 12-hr, light-dark cycle.

Apparatus

A wooden grey-painted T maze 17 cm high and 8 cm wide was used to monitor locomotor activity, spontaneous alternation, and food-reinforced alternation. Start box, stem, and each of the arms were 27, 66, and 39 cm long, respectively. A food well 1.5 cm in dia. was carved on a wooden platform 2 cm high at the end of each arm. Guillotine doors were fitted at the thresholds of the start box and each of the two arms. A mesh hardware cloth lid covered the maze, which was illuminated by a 15 W bulb placed 50 cm above the choice point.

Six photoactometer cages (BRS Foringer, No. PAC-001) each 61 cm in dia., with grid floors and black walls 43 cm high were used to examine both basal locomotor activity and the locomotor response to d-amphetamine. The interior of the cages was crossed by six infrared photocell beams, interruption of which incremented electromechanical counters. An automatic printout counter (BRS Foringer, No. POS-112) cumulated photobeam interruptions over periods of 10 min. Four 100 W bulbs were located 3.6 m above the cages.

Procedure

Surgical. Seven days after their arrival, the rats were randomly assigned to either a control or a kainic acid-lesioned (KAL) group, with 10 rats in each. The rats were anesthetized with sodium pentobarbital (50 mg/kg IP) and positioned on a Kopf stereotaxic instrument, with the incisor bar adjusted at 4.2 mm below the interaural line. Two holes were drilled in the skull, and 3 nmoles of KA dissolved in 0.5 μl of a phosphate buffered isotonic saline solution, pH 7.2, were bilaterally injected over a 3-min period at the following coordinates: 9.6 mm rostral and 4.5 mm dorsal to the interaural line, 2.8 mm lateral to the sagittal suture. In pilot experiments these coordinates were shown to correspond approximately to A=8.4, H=0.8, and L=2.2 of the König and Klippel atlas [21]. After the injection, the 34 gauge cannula was left in place for 5 min to prevent upward movement of the solution to the neocortex along the cannula track. The control rats were bilaterally injected with 0.5 μl of the vehicle solution only.

Behavioral. Three weeks after surgery a food deprivation schedule was instituted, in which the rats were given about 12 g of Purina Rat Chow daily until they reached 85% of their free-feeding weight. The amount of food was later adjusted to allow an average daily increase of 1.5 g in body weight. The rats were given food 1 hr after the time of day at which they were later tested. The rats were handled for eight daily periods of 5 min each before the behavioral experiments took place.

On daily Sessions 1–5 locomotor activity in the maze was examined. The rats were placed at the choice point and the entries into the various sections of the maze, including the start box, the stem, and each of the arms, were recorded during each of five 1-min periods. Three-way analysis of variance with repreated measures on two Factors, Sessions and Periods, was conducted on the entries [53].

On daily Sessions 6–10 the rats were first given a trial to establish their arm preference. Then they were given two trials of spontaneous alternation. On each trial the rats were placed in the start box, and the door was opened after 5 sec. After the rat entered an arm, it was confined there for 10 sec, and was then transferred to a 20×20×20 cm waiting box for a 20 sec intertrial interval (ITI). If a rat did not enter any arm within 300 sec from the onset of the trial, it was considered to have failed to make a choice in that and the following trials of the session. Rats that failed to make all choices in the five sessions were given a make-up session on Day 11. Differences between groups in number of choices and in percentages of spontaneous alternation in the first or the second alternation trial of all sessions combined were evaluated using nonparametric, Mann-Whitney U statistic [49].

On Session 12 the rats were adapted to eat 45 mg Noyes pellets in the maze. On Trial 1 ten pellets were scattered on the floor of the maze, four of which were in the stem, two in each arm, and one in each food well. On Trial 2, two pellets were placed in each arm, and two in each food well. On Trial 3, four pellets were placed in each of the food wells. In each trial, if a rat failed to eat all pellets within 5 min, it repeated that trial until the criterion of eating all pellets in 5 min was met. An ITI of 20 sec was used.

On daily Sessions 13–37 the rats were first given a trial in which four pellets were present in both food wells. In each of the subsequent five alternation trials four pellets were only present in the food well of the arm opposite to that chosen in the previous trial. Durations of confinement in the start box and the goal box, and the ITIs were identical to those used in the sessions of spontaneous alternation. The rats were trained to a criterion of 18 alternations in four consecutive sessions, or for a maximum of 25 sessions. Differences between groups in both errors and sessions to criterion were evaluated using Mann-Whitney U statistic.

After completion of the alternation experiment, the rats were given free access to food and water for one week. Locomotor activity in the photocell cages was then examined. The rats were individually placed in the photoactometers at about 11 a.m. and their activity recorded for six periods of 10 min each. They were then injected with 1 mg/kg of d-amphetamine IP, and immediately replaced in the photoactometers for another twelve 10-min periods. Two-way analyses of variance with repeated measures on Periods [53] were separately conducted on the pre- and the post-injection activity scores.

Histological. After completion of the beahvioral experiments the rats were deeply anesthetized with ether and perfused intracardially with isotonic saline solution followed by

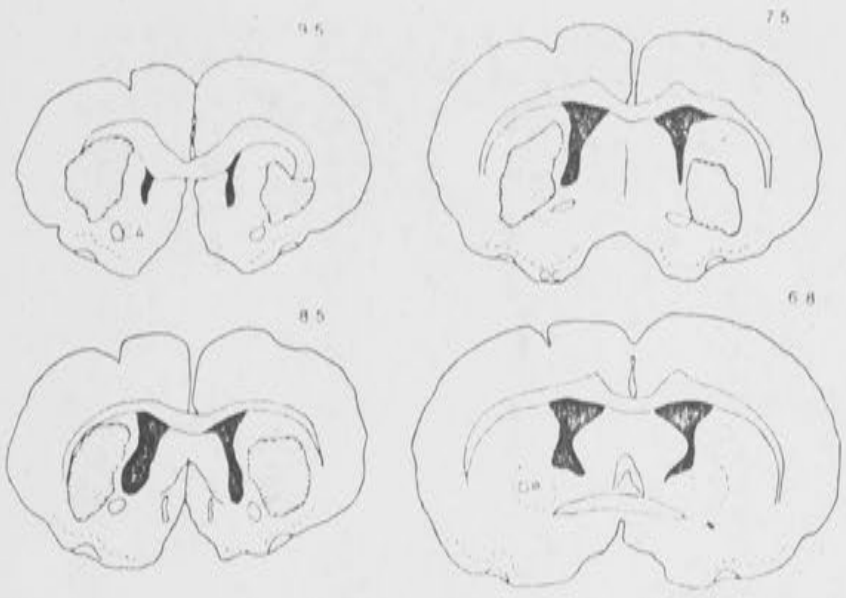

FIG. 1. Reconstruction of volume of neuronal loss (hatched areas) in the striatum of a representative rat with bilateral injections of 3 nmol/0.5 μl of kainic acid. Numbers correspond to frontal planes of König and Klippel atlas [15]. CA = anterior commissure; GP = globus pallidus; OF = olfactory cortex.

perfusion with 10% formaldehyde in saline solution. The brains were extracted and stored in 10% formaldehyde solution for two weeks. Frontal sections were cut from frozen tissue at 50 μ and alternatively stained either with cresyl violet or with the Kluver-Barrera method [19] for identification of both cell bodies and myelinated fiber bundles. The cortical and nuclear structures of the forebrain were examined in light microscopy for qualitative assessment of the kainate-induced pathological alterations.

RESULTS

Histology

In all KAL rats the forebrain ventricles were markedly enlarged and both striata were severely shrunken. The striatal lesions were usually ovoidal, with the center approximately at A = 8.0–8.4 and with rostro-caudal diameter of 2.0–2.5 mm (Fig. 1). The core of both the dorsal and the ventral striata was almost completely devoid of neurons, but a rim of apparently normal neurons remained in the dorsomedial and ventrolateral periphery. Fiber bundles of the internal capsule traversing the striatum appeared to be more packed than in normal tissue, but their size and stain density were not appreciably altered (Fig. 2). The space around the fiber bundles was densely infiltrated with glial cells. The globus pallidus showed a similar gliosis, but most of the large neurons were spared.

In addition to the striatal lesions, the brains of eight KAL rats revealed lesions in the hippocampus (Fig. 3). Specifically, partial bilateral neuronal loss was found in the hippocampal cell fields CA1 and CA3 of KAL rats 2 and 4. Partial neuronal loss in the CA1, but not in the CA3 fields, was found in KAL rats 7 and 8, bilaterally, and in KAL rats 5 and 9, unilaterally. In addition to the hippocampal damage, KAL rats 2, 4 and 9 showed a moderate neuronal loss and increase of glia in the deeper layers of the neocortex above the injection site. Bilateral neuronal loss and glial infiltration were found in the lateral hypothalamus of KAL rats 5 and 6, bilaterally, and of KAL rat 3, unilaterally. With the qualitative methods of examination used in this study, no additional damage could be confidently detected in the forebrains of the kainate-treated rats.

The brains of the control rats showed a slight glial infiltration along the cannula track, but they were otherwise intact.

Behavior

Postoperative recovery. Soon after recovery from anesthesia all KAL rats showed clonic jerks of the forelimbs, circling and body tremor for about 3–5 hr. In the following days all KAL rats, including those with no apparent hippocampal or hypothalamic damage, manifested aphagia, adipsia, and little or no grooming. After tube-feeding with Soyalac® for 3–4 days the KAL rats resumed spontaneous feeding and drinking. Four KAL rats showed bleeding from the urogenital tract for 4–5 days after surgery.

Chronic motor disorders were then observed in most KAL rats, including excessive abduction of the limbs and paws during walking, and unusually forceful struggling and body tremor during handling. Tonic-clonic seizures were witnessed in KAL rats 2, 3, 4, 6, 7, 8 and 9 from 37 to 77 days after surgery, usually in the course of the daily sessions of instrumental alternation. The rats showed only one seizure episode in each of the daily observation periods which lasted approximately 10 min. Two KAL rats exhibited seizures once, another two twice, and the remaining rats in three, six and seven sessions, respectively. The seizures lasted 25–30 sec and had a similar motor progression to that of class 4–5, kindling-induced seizures [40]. With the possible exception of food deprivation, no common precipitating condition could be identified, since the seizures occurred in different places, including the maze, the waiting box, and the home cages. There was no straightforward correlation between seizures and hippocampal lesions: KAL rat 3, which showed seven episodes of seizures, had no detectable hippocampal damage. KAL rats 1 and 5, in which seizures were never witnessed, had bilateral hippocampal lesions. It is of course possible, however, that the latter two rats had seizures at times in which they were not observed. In addition to the recurrent tonic-clonic seizures, KAL rats 4, 7 and 8 showed recurrent episodes of body circling and tail biting. These stereotyped behaviors were also exhibited by KAL rat 5, in which tonic-clonic seizures were never observed.

Locomotor activity in the T maze. Figure 4 shows the mean locomotor activity of the two groups in each 1-min period of five daily sessions. The rats of both groups showed a progressive decrease of activity with time, as indicated by the significant effect of periods, $F(4,72) = 1.6$, $p < 0.001$. The main effect of KA treatment did not reach statistical significance, $F(1,18) = 1.6$, $p > 0.1$, but the Treatment × Sessions interaction was significant, $F(4,72) = 2.8$, $p < 0.05$. Newmann-Keuls tests [53] comparing the activity of the groups in each session revealed that the KAL rats entered significantly less compartments than the controls in Session 1, $p < 0.05$, but not in the subsequent sessions. Although the Treatment × Periods interaction did not reach statistical significance, $p > 0.1$, Newmann-Keuls tests comparing the activity of the groups in each 1-min period of all sessions combined showed that the KAL rats entered significantly less compartments than the controls in the first period, $p < 0.05$, although not in the subsequent periods. Examination of the activity scores of each KAL rat either in the first session or in the first 1-min period of all sessions combined did not reveal appreciable differences between

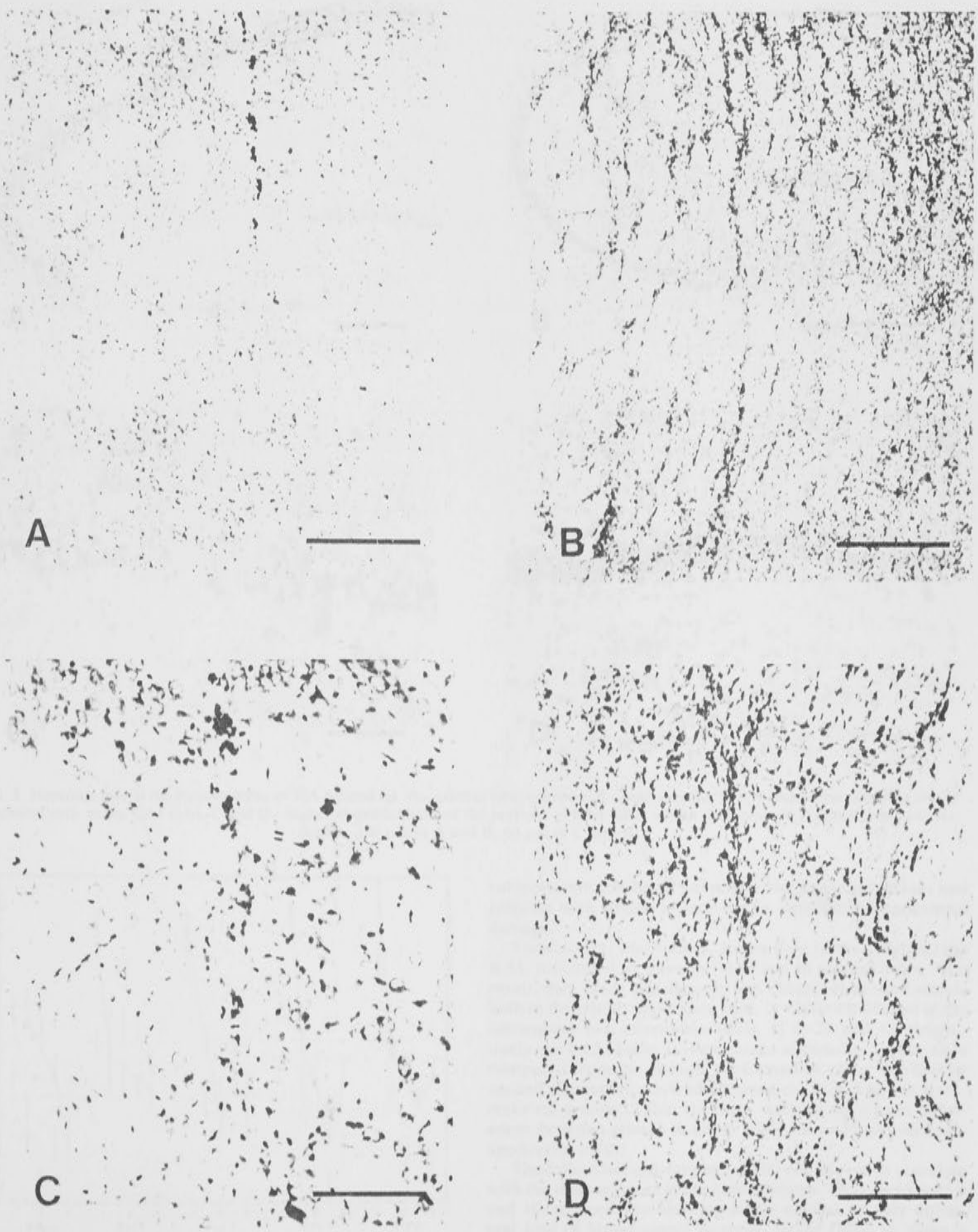

FIG. 2. Sections through striatal injection sites. A—striatum of control rat. B—striatum of KA-treated rat, showing absence of neurons, marked glial proliferation and spared fiber bundles, which, however, appear more packed than in control tissue. C and D—higher magnifications of injection sites shown in A and B, respectively. Scales, 200 μm in A and B, 80 μm in C and D.

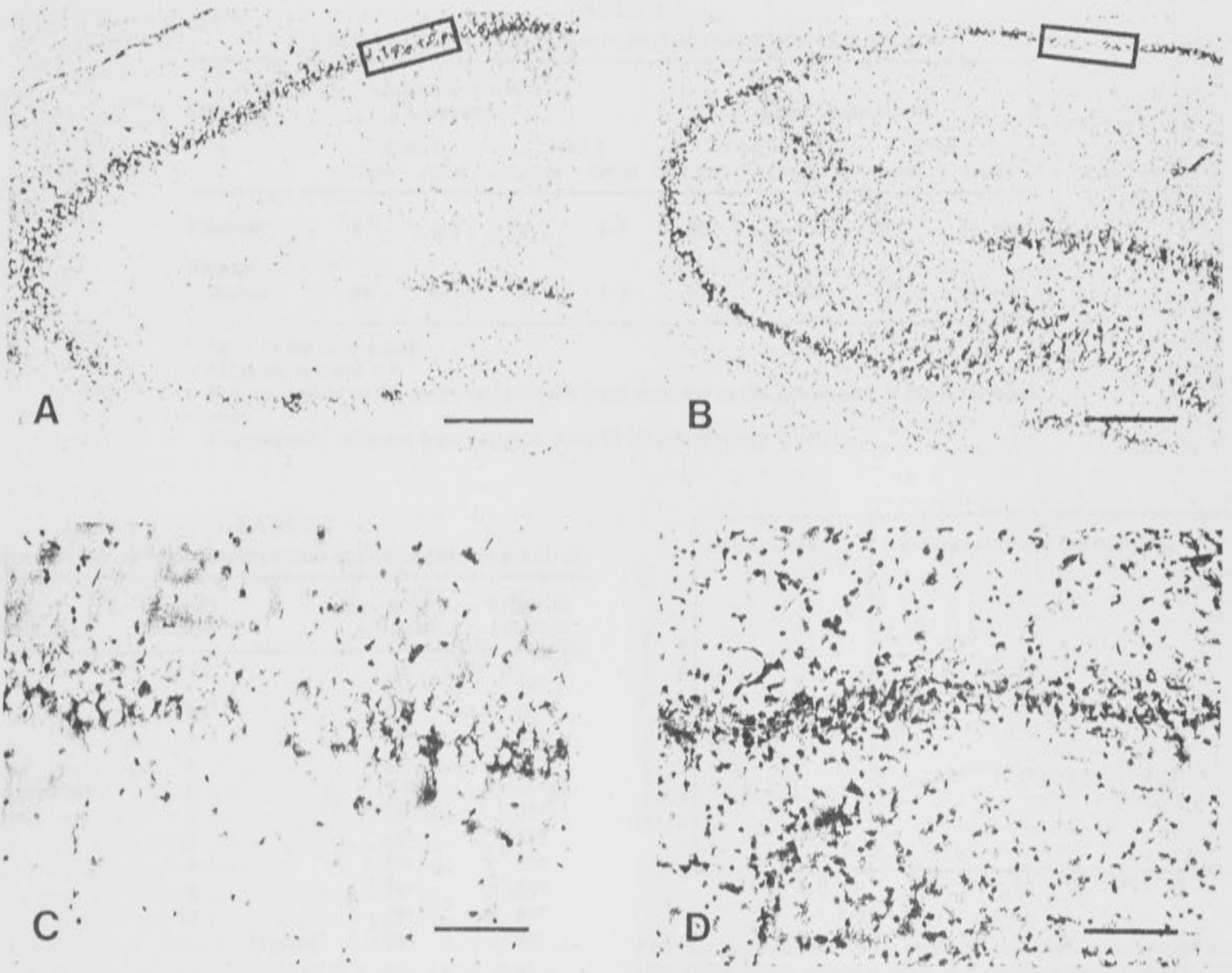

FIG. 3. Neuronal loss in the hippocampus of KA-treated rat. A—control hippocampus. B—hippocampus of KA-treated rat, showing loss of pyramidal cells in the CA1 field. C and D—higher magnifications of the portions of CA1 fields within the frames in A and B, respectively. Scales, 250 μm in A and B, 60 μm in C and D.

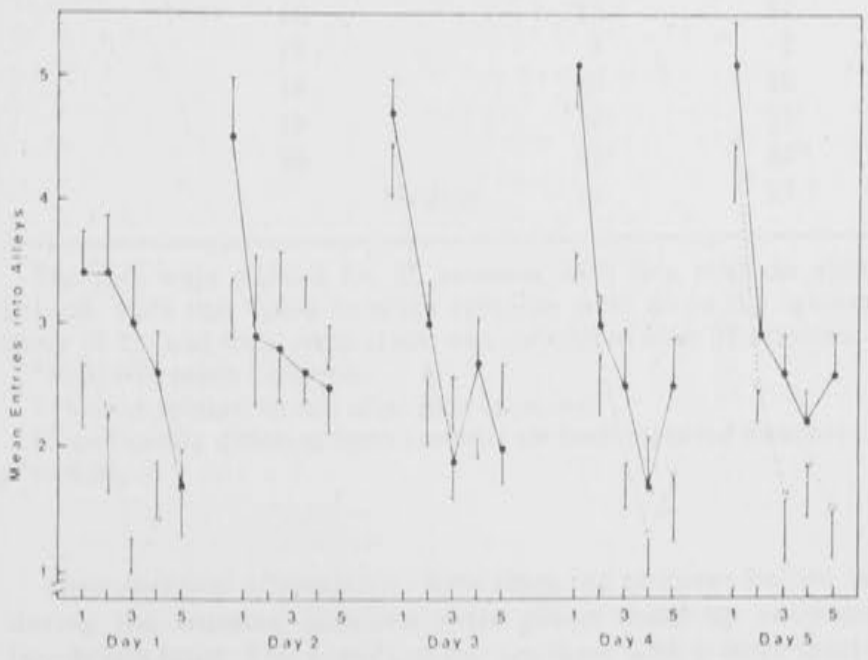

FIG. 4. Locomotor activity in the T maze. Filled circles=control rats; open circles=KA-treated rats. Numbers in abscissa indicate 1-min periods. The end bars represent ± SEM.

subjects with combined striatal and hippocampal lesions and subjects with striatal lesions but no detectable hippocampal damage.

Spontaneous alternation. In contrast to the controls, the KAL rats failed to leave the start box in several trials. As a result, they made significantly less choices than the controls both in the initial, preference trial, U=20, $p<0.05$, and in the subsequent two alternation trials, U's=22 and 12, respectively, $p<0.05$ (Table 1). Percentage alternation scores were computed from the choices performed in either the first or second alternation trials of all sessions, including the sixth, make-up session (Table 1). There were no significant differences between groups in either trials, U's=35 and 46.5, respectively, $p>0.1$.

The failure to perform choices did not appear to correlate with the neuronal loss in the hippocampus. Thus, KAL rats 9 and 10, respectively showing either no loss or only unilateral loss of hippocampal neurons, never failed to make a choice. One the other hand, KAL rats 3 and 5, although also showing either no loss or only unilateral loss of hippocampal neurones, had the lowest choice scores in the group.

TABLE 1

CHOICE BEHAVIOR AND SPONTANEOUS ALTERNATION IN A T MAZE

Group*	Choices in the first 5 sessions†				Percent alteration‡			
	Trial 1		Trial 2		Trial 1		Trial 2	
	median	range	median	range	median	range	median	range
Control	5	4–5	5	4–5	60	40–100	80	60–100
Striatal Kainic	4§	1–5	3§	1–5	60	0–100	87.5	20–100

*n = 10 for each group.

†Maximum score = 5.

‡Choices made in the make-up session 6 were included in the calculation of the percentage scores.

§Significantly different from controls, $p<0.05$ (Mann-Whitney U test).

TABLE 2

ACQUISITION OF FOOD REINFORCED ALTERNATION IN A T MAZE

Group	Subject number		Sessions to criterion	Errors to criterion
Neostriatal§ Kainic	1		25*	39*
	2		25*	66*
	3		25*	65*
	4		25*	72*
	5		—†	—†
	6		25*	73*
	7		25*	85*
	8		25*	77*
	9		25*	125*
	10		25*	84*
		Median	25	73
Control	11		12	21
	12		19	23
	13		25	38
	14		23	33
	15		4	2
	16		16	24
	17		5	3
	18		18	20
	19		16	23
	20		25*	44*
		Median	18	23.5

The rats were trained for 25 sessions with five trials in each session. Rats that failed to reach criterion were given the session score of 25, and their error score was calculated over 25 sessions.

*Failed to reach criterion.

†This rat refused to run after four sessions.

§Significantly different from controls on both reported measures, p's<0.01.

Instrumental alternation. Rats showing seizures before or during the training sessions were given make-up sessions two hours later. The scores of the sessions which were interrupted due to seizures were excluded from the statistical analyses. After four sessions KAL rat 5 repeatedly failed to leave the start box and it was therefore excluded from the experiment. Except for this rat, all KAL rats consistently left the start box and reached the goal box after food was introduced in the maze. The KAL rats however showed clearcut impairments in performance of instrumental alternation; nine control rats reached the criterion of 18 alternations in 20 consecutive trials, none of the KAL rats did so (Table 2). There were significant differences between groups on both errors and sessions to criterion, U's=5 and 9, respectively, nl/n2=9/10, $p<0.01$. The failure in instrumental alternation did not appear to depend only on the presence of hippocampal damage, because the error scores of KAL rats 3 and 10, which had no detectable hippocampal damage, and of KAL rat 9, which had only unilateral hippocampal damage, were not lower than those of rats with bilateral hippocampal degeneration.

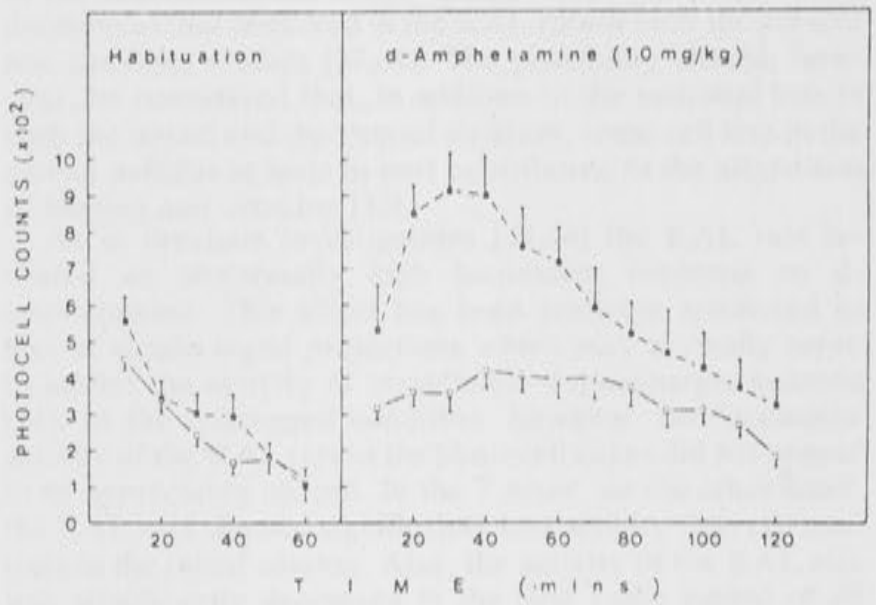

FIG. 5. Locomotor activity in photocell cages before (left panel) and after (right panel) administration of d-amphetamine (1 mg/kg, IP). Filled squares = control rats; open squares = KA-treated rats. The end bars represent ± SEM.

Spontaneous and d-amphetamine induced locomotor activity. The results of the locomotor tests in the photocell cages are shown in Fig. 5. Analysis of the pre-injection activity scores revealed a significant effect of periods, $F(1,18)=35.4$, $p<0.01$, reflecting progressive habituation. Neither the treatment effect, $F<1$, nor the interaction,

$F(5,90)=1.7$, were significant, $p>0.1$. After d-amphetamine administration the rats of both groups increased their motor activity, but the KAL rats did so significantly more than the controls, $F(1,18)=8.1$, $p<0.02$. The difference between groups was especially marked in the first hour, as indicated by the significant interaction of KA treatment with periods, $F(11,198)=7.2$, $p<0.001$.

DISCUSSION

The present histological data agree with previous reports [5, 6, 9, 15] that striatal injections of KA destroy neuronal cell bodies but do not grossly alter the morphology of fiber bundles traversing the injected area. In addition to striatal degeneration, however, loss of neurons was usually found in the hippocampus, and, in a few cases, in the neocortex above the injected area and in the lateral hypothalamus.

Although one group of investigators [6] reported extrastriatal lesions only with doses of KA as large as 9.5 nmoles, others have found extrastriatal damage with much smaller doses. Thus, Olney and de Gubareff [36] reported degeneration of the prepyriform cortex and the amygdaloid area after injections of 2.5–5 nmoles/μl in the central striatum, although not after injections of smaller volumes and doses in the dorsal striatum.

Brain lesions of variable extent have been found after systemic injections of identical doses of KA [48]. Our experience suggests that a similar variability can also occur after intracerebral injections of this neurotoxin, because using identical injection procedures to those of the present study, we have obtained relatively small striatal lesions with little or no detectable extrastriatal damage in some series of KAL rats, and relatively large striatal lesions with considerable extrastriatal damage in others (Pisa, Sanberg and Fibiger, unpublished observations).

There is evidence that several factors can influence the neurotoxic effectiveness of intracerebral injections of KA, including dose, volume, infusion rate and stability of the solution [27], rat strain [45], and the nature and amount of anesthetic [34]. Although differences in the use of one or more of these parameters can at least in part account for the discrepant results of different laboratories, they presumably do not account for the variable effects obtained in this laboratory, since these parameters were always kept constant. It has been noted that bottles of KA from the same manufacturer (Sigma) may differ in neurotoxic potency ([27]; Meibach, personal communication). It may also be speculated that differences in susceptibility to KA may exist not only between strains [44] but also between litters of the same strain. Whatever the reasons for the variable effectiveness of KA, it is apparent that even relatively small doses of this neurotoxin may fail to produce lesions that are confined to the injected structure. In view of these results, future studies should provide histological documentation of the effect of KA injections that is not limited to the effects in the injected area.

The behavioral alterations of the KAL rats included temporary aphagia, adipsia, and lack of grooming, alterations of gait and locomotor activity, convulsive and stereotyped behaviors, and learning impairments.

The findings of temporary aphagia and adipsia in KAL rats confirm those of other studies [37,42] in which a long lasting decrease of body weight was also found in rats with intrastriatal injections of KA. Aphagia and adipsia of variable duration have been reported after coagulative lesions of the neostriatum [54], globus pallidus [32], and lateral hypothalamus [51], after ablations of the orbitofrontal cortex [20], after 6-hydroxydopamine-induced degeneration of nigro-striatal dopaminergic fibers [1, 11, 23, 52], and after KA injections in the lateral hypothalamus [14]. In the present study, none of the KAL rats had detectable lesions in the orbitofrontal cortex, and only three of them had appreciable lesions in the lateral hypothalamus. We previously confirmed [44] the original finding [7,26] that striatal injections of KA do not decrease local concentrations of dopamine, suggesting no appreciable damage to nigrostriatal dopaminergic fibers. Also, lesions of the hippocampus do not result in aphagia and adipsia [17]. On the basis of this evidence, it would appear that damage to either extrastriatal neurons or to the dopaminergic innervation of the striatum cannot account for the aphagia and adipsia of the KAL rats. The interpretation that the alterations in food and water intake resulted from loss of striatal neurons is consistent with previous investigations[54] showing that electrolytic lesions of the rostral caudate cause aphagia and adipsia of similar duration to that observed in the KAL rats of both the present and previous studies [37,44]. The possibility should, however, be considered that, in addition to the neuronal loss in both the dorsal and the ventral striatum, some cell loss in the globus pallidus at least in part contributed to the alterations of feeding and drinking [32].

As in previous investigations [24,44] the KAL rats revealed an abnormally high locomotor response to d-amphetamine. This effect has been tentively attributed to loss of striato-nigral projections which may normally serve to inhibit the activity of mesolimbic dopaminergic neurons [24]. In the undrugged condition, however, the locomotor activity of the KAL rats in the photocell cages did not appear to be appreciably altered. In the T maze, on the other hand, the KAL rats showed significantly less activity than the controls in the initial session. Also, the activity of the KAL rats was significantly decreased in the first 1-min period of all sessions combined. It should be noted that, in contrast to the scoring method used in the T maze, the activity scores in the photocell cages were cumulated over 10-min periods. This method might have failed to reveal a decrease of locomotor activity in the KAL group at the onset of the first 10-min period.

The apparent reluctance of the KAL rats to initiate ambulatory behavior was confirmed in the test of spontaneous alternation, in which, unlike the controls, several KAL rats failed to leave the start box on many trials. Nonetheless, it is apparent that both in the photocell cages and in the T maze, the locomotor activity of the KAL rats was similar to that of the controls a few minutes after the onset of testing. The initial hypoactivity of the KAL rats thus appears to be suggestive of an increased freezing reaction to novel environments rather than a generalized hypokinesia. This specific locomotor alteration has not been reported in previous studies of locomotor activity in rats with coagulative lesions of the striatum [18, 33, 50, 54]. However, it appears from these studies that, in addition to the site and size of the lesions, the amount of locomotor activity in rats with striatal lesions can be influenced by interactions of these lesions with such factors as sex, method of measuring activity, illumination, dietary schedule, and circadian cycle. Differences in one or more of these factors could thus account for the conflicting reports of decreases [33], increases [18,54] or no changes [50] of locomotor activity in rats with striatal lesions.

Although the interpretation of the locomotor alteration found in the present study is complicated by the occurrence of hippocampal degeneration in several KAL rats, there appeared to be no consistent correlation between this behavioral effect and extent of extrastriatal damage. Furthermore, similar behavioral effects have subsequently been found in KAL rats with no apparent extrastriatal damage (Pisa, Sanberg, and Fibiger, in preparation). Taken together, these results suggest that loss of striatal neurons can interfere with onset of exploratory behaviors, possibly as a result of increased neophobia.

The KAL rats showed a severe impairment in performance of instrumental left-right alternation. A generalized impairment in learning instrumental responses does not appear to account for this result, because KAL rats show no alterations in learning simple responses, such as bar pressing [44]. Since the alternation task presumably involves learning to use memories of recent responses, it might be suggested that the lesions interfered with short-term memory. Nonetheless, the KAL rats did not appreciably differ from the controls in rate of spontaneous alternation. Since spontaneous alternation presumably also involves memory of recent responses [10], it would appear that the kainate-induced lesions did not result in a generalized failure of short-term memory. Compared with the test of spontaneous alternation, the food-reinforced alternation task involved both a larger number of trials in each session and the availability of a highly arousing stimulus, i.e. food. It might be speculated, therefore, that either an abnormally high arousal reaction to appetitive stimuli or an increased susceptibility to interference resulting from accumulated trials, or both, accounted for the failure of the KAL rats. Divac *et al.* [9] recently found that rats with KA-induced striatal lesions were impaired in postoperative retention and relearning of left-right alternation although not of visual discrimination. They specifically attributed the alternation impairment to loss of striatal neurons. Unfortunately, no mention was made of the possible contribution of extrastriatal neurodegenerative effects of KA injections. Indeed, neuronal loss in extrastriatal structures, including the hippocampus, was later found (Divac, personal communication) in rats with identical KA striatal injections to those used in Divac *et al.* study [9]. In view of the evidence that hippocampal ablations in rats impair performance of spatial alternation [30, 38, 39] it is possible that both in the present study and in that of Divac *et al.* [9] the failure of at least some KAL rats resulted partly from loss of hippocampal neurons. In the present study, however, KAL rats with either no hippocampal damage or partial, unilateral loss of hippocampal neurons were just as impaired as those with bilateral hippocampal degeneration, indicating that striatal degeneration can by itself result in failures of alternation performance.

It has already been reported that rats with systemic or intracerebral injections of KA can exhibit clonic seizures soon after recovery from anesthesia [36,55]. The present observations of recurrent tonic-clonic seizures in KAL rats even months after the injections is a new finding, and suggests that kainic acid might be used to reproduce a model of chronic epilepsy.

Impairments of discrimination learning have been reported in animals with chronic electrocorticographic alterations resulting from implantations of antibiotics in various forebrain areas [16]. Although some of the KAL rats that failed to perform the alternation task did not exhibit motor seizures, it is possible that these rats, like those with demonstrated tonic-clonic seizures, had chronic alterations of brain electrical activity which might, at least in part, account for their behavioral impairments.

In summary, the present study shows that neuronal degeneration induced by striatal injections of kainic acid in rats can result in a wide range of behavioral disorders, including temporary aphagia and adipsia, alterations of gait, stereotyped behaviors such as circling and tail biting, acute and chronic epileptic seizures, delayed onset of ambulatory behavior, and impairments in performance of instrumental spatial alternation. In agreement with the proposed similarity between KA striatal model and Huntington's disease, several of these behavioral effects, including the regulatory, motor and problem solving impairments appear to be similar to those usually found in HD patients. Although correlations between behavioral and neuropathologic effects of kainate injections suggested that degeneration of the striatum was mainly responsible for the behavioral impairments, a possible contribution of the attendant extrastriatal lesions cannot be excluded. If kainic acid is to be continued to be used for detailed neuropsychological investigations, a better understanding of the factors responsible for the variability and multifocality of the degenerative effects of this neurotoxin is clearly needed.

ACKNOWLEDGEMENTS

The authors appreciate the excellent and dedicated assistance of S. Atmadja. This work was supported by the Medical Research Council. M. Pisa is an MRC Fellow.

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STRIATAL INJECTIONS OF KAINIC ACID SELECTIVELY IMPAIR SERIAL MEMORY PERFORMANCE IN THE RAT.

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to be published in

Experimental Neurology

ABSTRACT

Rats with bilateral injections of kainic acid into the striatum were trained on a schedule of either singly alternated or continuous reinforcement in a runway. Both the acquisition and the extinction rates of the kainate-treated rats did not significantly differ from that of control rats with either reinforcement schedule. However, the kainate-treated rats ran significantly more slowly than the controls, especially at the onset of the training sessions, and, in contrast to the controls, failed to show reliable speed alternation in the late trials of sessions with reward alternation, thus indicating both a locomotor impairment and an impairment of serial memory performance. In addition to severe loss of striatal neurons, the kainate injections induced partial neuronal loss in the neocortex, globus pallidus, hippocampus, and pyriform cortex. The similarity of the kainate-induced behavioural and pathological alterations to those of Huntington's disease is discussed.

Key words: Kainate. Striatum. Huntington's disease. Locomotion. Alternation. Extinction. Temporal discrimination. Memory.

Injections of kainic acid (KA) into the rat neostriatum cause a marked loss of intrinsic neurons, apparently leaving largely intact the cortical afferent and efferent fibers that pass through the striatum (9, 10, 12, 25). Both the interpretative advantage resulting from the use of this relatively selective axon-sparing method of lesions, and the similarity of the KA-induced striatal pathology with that of Huntington's disease (HD) have prompted several investigators to re-examine the behavioral functions of the striatum using the KA-lesioning technique (12, 18, 22, 23, 29, 32, 33). In these studies kainate injections into the rat striatum were shown to impair both locomotor behaviors and learning and memory performance. The present experiments were conducted to further analyse the nature of these behavioral alterations.

EXPERIMENT 1

Coagulative lesions of the rat neostriatum have been shown to impair performance of delayed, left-right alternation (DLRA) tasks (25, 27). More recent investigations revealed similar impairments of both acquisition (29) and postoperative retention and relearning (12) of DLRA tasks in rats with KA-induced lesions of the striatum (KAL rats). The nature of the functional alterations underlying these behavioral impairments remains to be elucidated, however. DLRA tasks are thought to involve use of both spatial cues, i.e. discriminating left from right, and temporal cues, i.e. memory, on each trial, of the choice made in the previous trial. Thus, although the proposed role of the striatum in egocentric localization (30) might suggest an interpretation of the DLRA impairment entirely in terms of a failure in spatial discrimination, it is possible that alterations of temporal discrimination also contributed to these impairments.

In view of the proposed similarity of KA-induced pathology with that of HD, a study of the effects of KA striatal injections on temporal discrimination appeared to be especially interesting, because HD patients

are impaired in serial memory tasks that do not involve spatial information processing (4).

A task of conditioned speed alternation was used to assess temporal discrimination performance. On practising a schedule of single alternation of reward and non-reward in a runway, normal rats learn to run more slowly on non-rewarded than on rewarded trials (2, 6, 8, 11, 39). There is substantial evidence supporting the interpretation (7) that use of temporal cues, specifically memory of the prevailing reward conditions in the previous trial, is the relevant source of stimulus control for this behaviour. On the other hand, spatial cues do not seem to play a significant role in conditioning of running speed. Thus, this task appeared to be well suited to assess the effects of KA striatal injections on temporal discrimination performance, independent of its possible effects on spatial discrimination.

METHOD

Subjects

Eighteen male Wistar rats (Woodlyn Farms, Guelph, Ontario) weighing 275-300gm at the time of surgery, were used. They were housed in individual stainless steel cages located in a colony room with temperature of 22-25^{o}C, humidity of 45% and a 12h light-12hdark cycle.

Surgery

Seven days after their arrival the rats were randomly assigned to a group with KA-induced lesions (N = 9) and a control group (N = 9). The rats were anaesthetized with intraperitoneal injections of sodium pentobarbital (50mg/kg) and positioned in a Kopf stereotaxic instrument, with the incisor bar adjusted at 4.2mm below the interaural line. Two holes were drilled in the skull, and three nmoles of KA (Sigma) dissolved in 0.5µl of a phosphate buffered isotonic solution, pH 7.2, were injected into the

striatum of the KAL rats over a 3min period at the following coordinates: 9.6mm rostral and 4.5mm dorsal to the interaural line, 2.8mm lateral to the sagittal suture. After the injection, the 34 gauge cannula was left in place for 5min to prevent upward movement of the solution to the neocortex. The injections were bilateral. The control rats were injected with 0.5µl of the vehicle solution only.

Apparatus

The L-shaped runway was 12cm wide, with wooden, gray-painted walls, 30cm high, and a mesh hardware cloth top. The start box was 35cm long, with a floor consisting of a hinged, wooden platform which operated a microswitch when it was depressed by the weight of the rat. The alley and the goal box were 132cm and 35cm long, respectively, with floor made of brass rods 0.5cm in diameter, set 2cm apart, center to center. The goal box was entered with a 90^{o} right turn from the alley, and had a guillotine door 5cm beyond its entrance, and a food cup 3cm in diameter and 2cm high attached to the floor 4cm in front of the endwall. The runway was equipped with three photocell beams, crossing the width of the runway 3cm above the floor. Two photocell beams were placed in the alley, 4cm and 124cm beyond the exit from the start box, respectively, and the third photocell beam was placed above the food cup. Relays connected the microswitch and the photocells to three timers, each calibrated in 0.1sec.

Procedure

The rats were allowed to recover from surgery for 21 days, during which they were given Purina Rat Chow food and tap water ad lib. Their daily amount of food was then limited to 12gm, until they reached 85% of their free-feeding weight, which took about a week, and it was later adjusted to allow an average daily increase of 1.5gm in body weight. The rats were always fed

1h after the time of the day in which they were later trained. Ten days after the onset of the food deprivation schedule the rats were handled for three daily periods of 5min each. At the end of each handling session, the rats were carried to their home cages and offered five, 45mg Noyes pellets twice, with an interval of 5min, 30min before being given the daily ration of food. The rats were then given three daily sessions of adaptation in the runway. On session 1, they were placed in the runway in groups of three and given the opportunity of free exploration for 20min. On sessions 2 and 3 they were individually placed in the start box, facing opposite the alley, and left in the runway for 5min or until they reached the food cup and ate the five 45mg pellets contained in it. If a rat failed to do so within 5min, it was carried to the goal box, confined there until it ate the food or for a maximum of 1min, and then given as many make-up trials as necessary until it reached the criterion of entering the goal box and eating the food in 5min for two consecutive trials.

The rats were then given daily sessions of training with alternating reward, each consisting of 12 trials, with a five-pellet reward being present in the food cup in the even-numbered trials and absent in the odd-numbered trials. Each trial started with the rat being placed on the platform of the start box, facing opposite the alley. When the rat reached the food cup, the guillotine door was lowered and the rat confined in the goal box for 15sec or until it ate the food. The rat was then carried to a waiting box, where it spent the intertrial interval (ITI). Acquisition training consisted of 22 daily sessions with a 1min ITI, followed by 10 sessions with a 5min ITI. Then the rat was given five daily sessions of extinction, each consisting of 12 non-rewarded trials, with a 5min ITI.

Measures of performance

Records were taken of the latencies in each segment of the runway. The startbox latency was the interval between the closure of the microswitch and the interruption of the first photocell beam. The alley latency was the interval between the interruption of the first and the second photocell beams. The goal box latency was the interval between the interruption of the second and the third photocell beams. If, on a given trial, the latency of the rat exceeded 60sec in any segment of the runway, the rat was directly carried to the goal box and confined there for the usual interval, with reward conditions appropriate to that trial.

Preliminary scrutiny of the data showed that cell means varied directly with their standard deviations, indicating a lognormal distribution of the data (35). Statistical analysis was therefore conducted on logarithmically transformed latency scores (logtimes).

Histology

After completion of training the rats were deeply anaesthetized with ether and perfused intracardially with isotonic saline solution followed by perfusion with 4% formaldehyde-saline solution. The brains were extracted and stored in the fixative for two weeks. Frontal sections were cut from frozen tissue at 40µ and alternatively stained with either cresyl violet or with the Kluver and Barrera (20) method for staining of both cell bodies and myelinated fiber bundles. Forebrain structures were examined by light microscopy for qualitative assessment of the KA-induced pathological alterations.

RESULTS

Anatomical

On macroscopic examination the brains of all KAL rats showed marked

bilateral shrinkage of the striatum and enlargement of the forebrain ventricles. The lesions (Figure 1) were usually ovoidal, and centered approximately at A 7.8 - 8.2, with reference to the König and Klippel atlas (21), and with rostro-caudal extent of 2.0 - 2.4mm, involving the dorsal striatum, bilaterally, in all KAL rats, and the ventral striatum, unilaterally, in KAL rats 31, 35 and 37. Histology of these areas (Fig. 2) showed almost complete loss of neuronal cell bodies, marked astrocytic infiltration, and sparing of the myelinated fiber bundles of the internal capsule. The rostral pole of the globus pallidus (Figure 2) revealed partial neuronal loss in KAL rats 30, 31 and 32, bilaterally, and in KAL rats, 35, 38 and 39, unilaterally.

Extrastriatal damage (Figure 3) included: 1) a slight but consistent decrease in the number of neurons in the juxtacallosal layers of the frontal cortex above the injection site, bilaterally; 2) foci of partial or complete neuronal loss in the hippocampus, usually involving a limited number of lamellae midway between the septal and temporal pole, and restricted to either the CA1 field, bilaterally in KAL rats 30, 31, 32 and 38, and unilaterally in KAL rats 34 and 35, or to the CA3 field, bilaterally in KAL rats 38 and unilaterally in KAL rats 34 and 35; 3) foci of partial or complete neuronal loss in the subamygdalar extent of the pyriform cortex in KAL rats 30, 31, 32 and 37, unilaterally. No damage to either the hippocampus or the pyriform cortex could be detected in KAL rat 37. Qualitative inspection of the thalamic, hypothalamic, septal, amygdaloid and accumbens nuclei failed to reveal appreciable astrocytic infiltration or neuronal loss in any KAL rat.

The brains of the control rats showed a slight glial infiltration along the cannula tract, but were otherwise intact.

Behavioural

Figure 4 shows the mean logtimes in the alley during training with

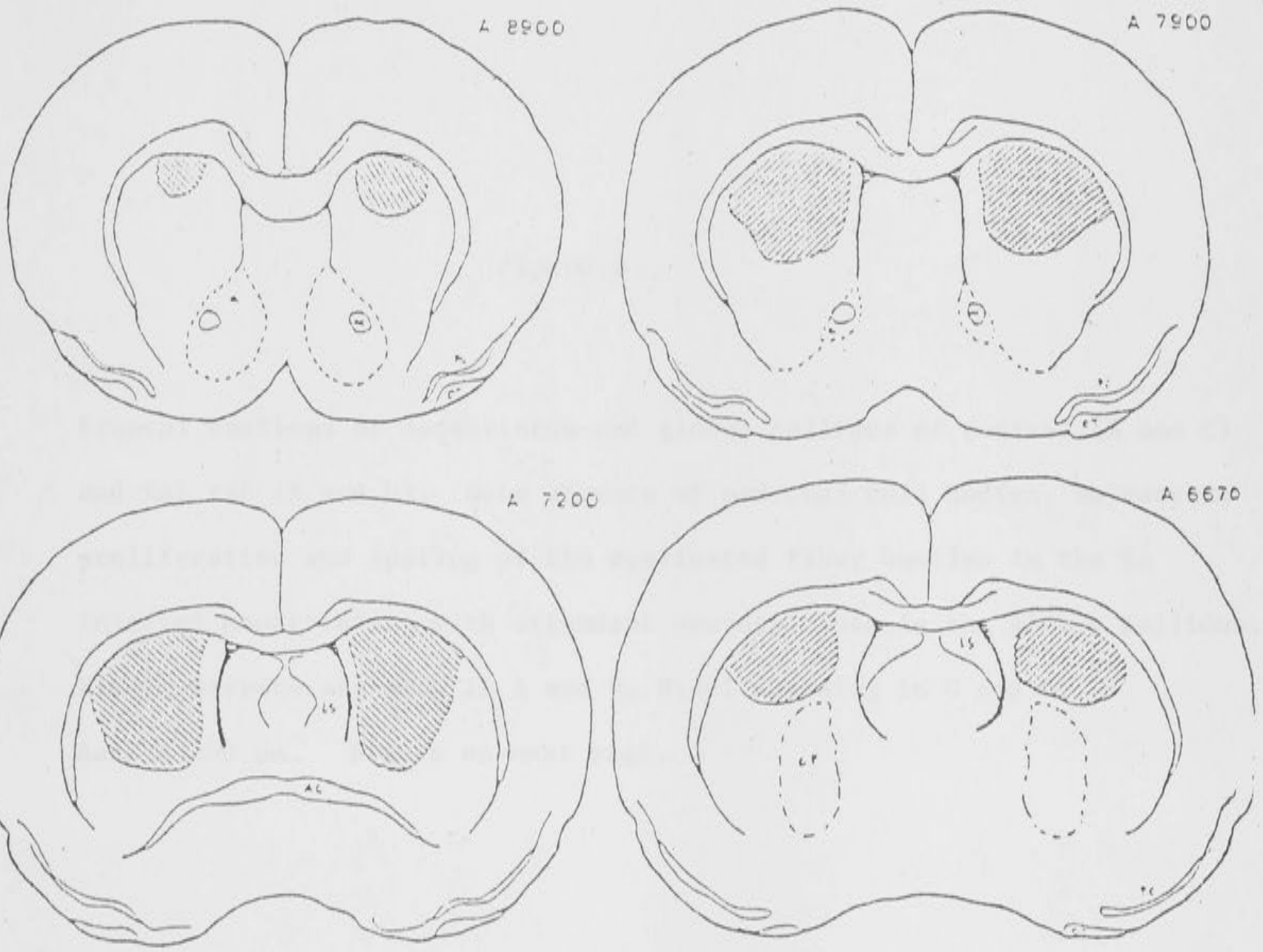

Figure 1. Reconstruction of volume of neuronal loss (hatched areas) in the neostriatum of a representative rat with bilateral injections of 3 nmoles of kainic acid (KAL rat). Numbers correspond to frontal planes of König and Klippel atlas. a = nucleus accumbens; AC = anterior commissure; GP = globus pallidus; LS = lateral septum; OT = optic tract; Pc = pyriform cortex.

Figure 2

Frontal sections of neostriatum and globus pallidus of control (A and C) and KAL rat (B and D). Note absence of neuronal cell bodies, astrocytic proliferation and sparing of the myelinated fiber bundles in the KA injected neostriatum, with attendant neuronal loss in the globus pallidus. Kluver-Barrera staining in A and B, Nissl staining in C and D. Bars = 200 μm. Figure on next page.

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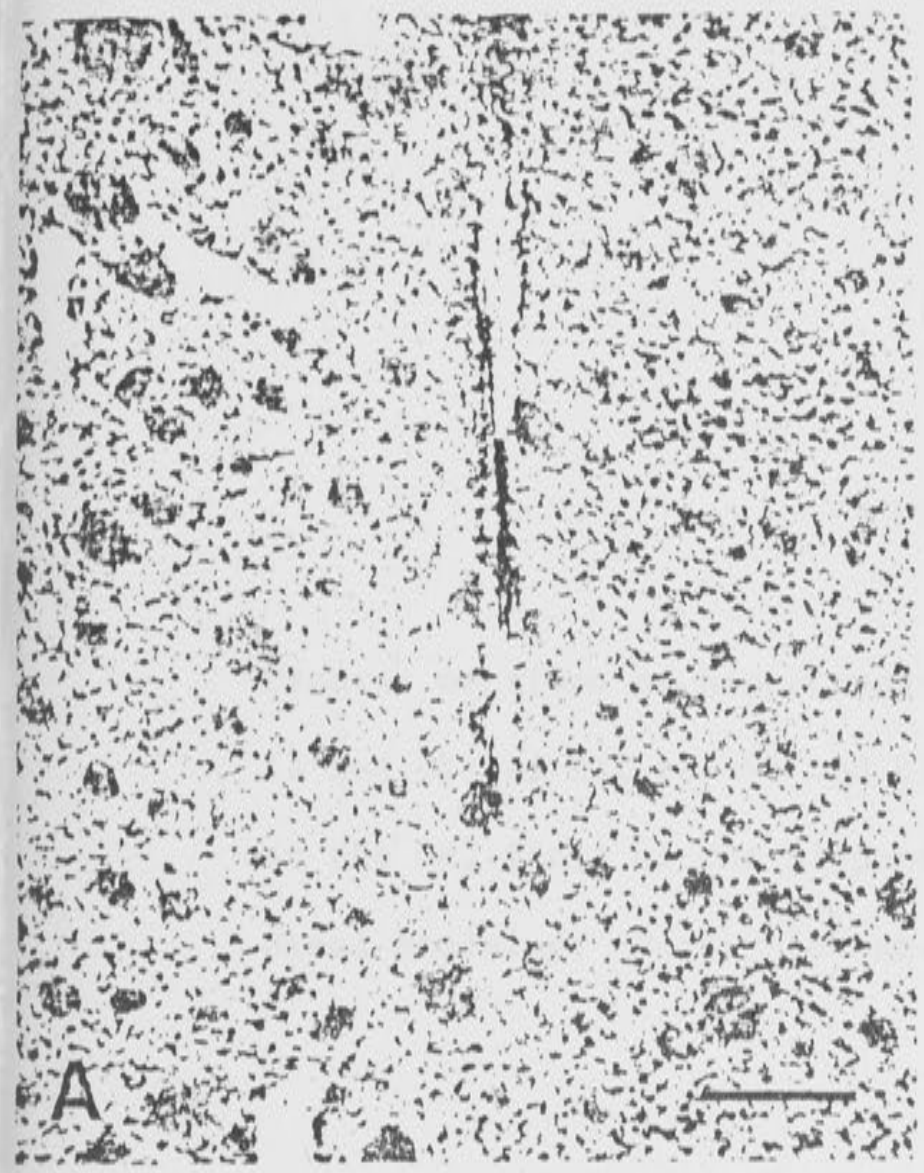
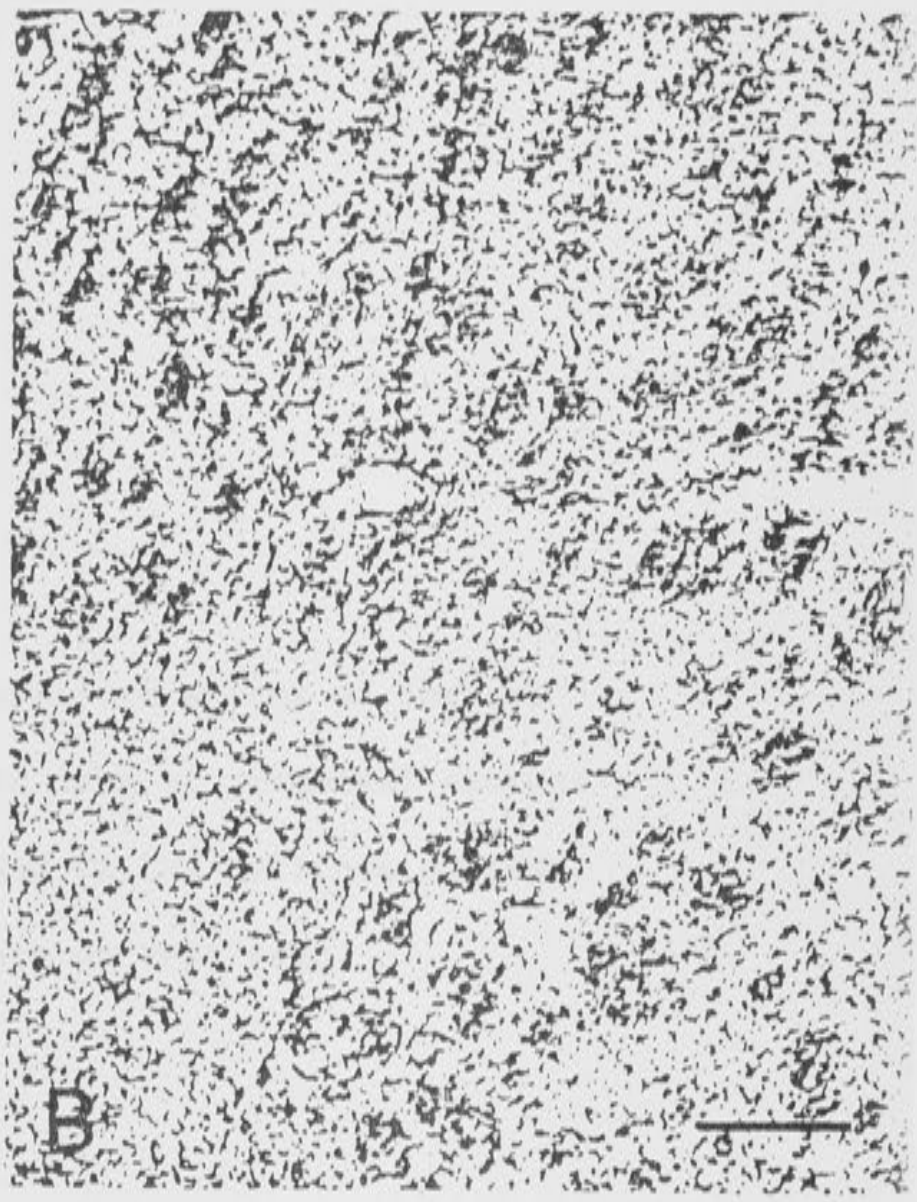

A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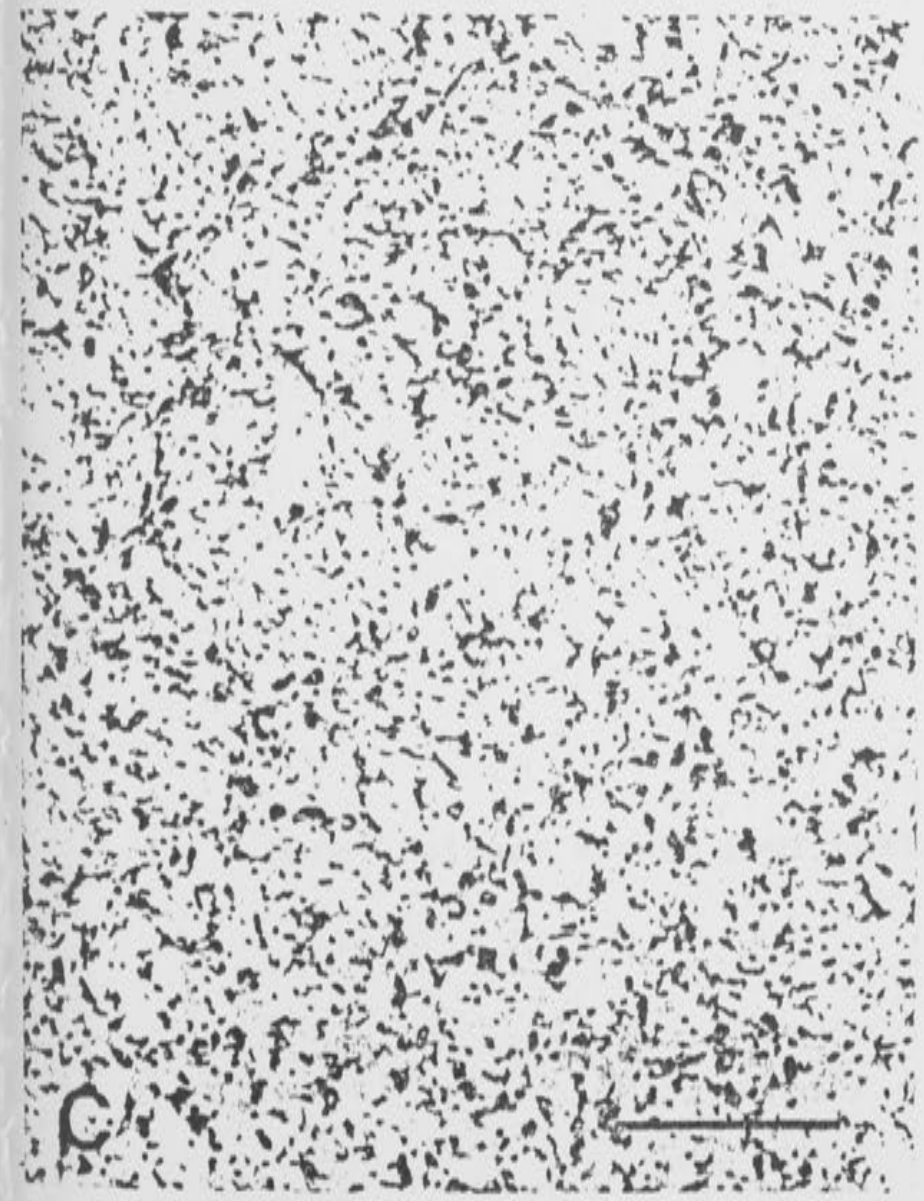
C


D

Figure 3

Frontal sections of hippocampus and pyriform cortex of control (A and C) and KAL rat (B and D). Note loss of pyramidal neurons in the CA3 field of the hippocampus and in the pyriform cortex. Nissl staining. Bars = 180 µm. Figure on next page.

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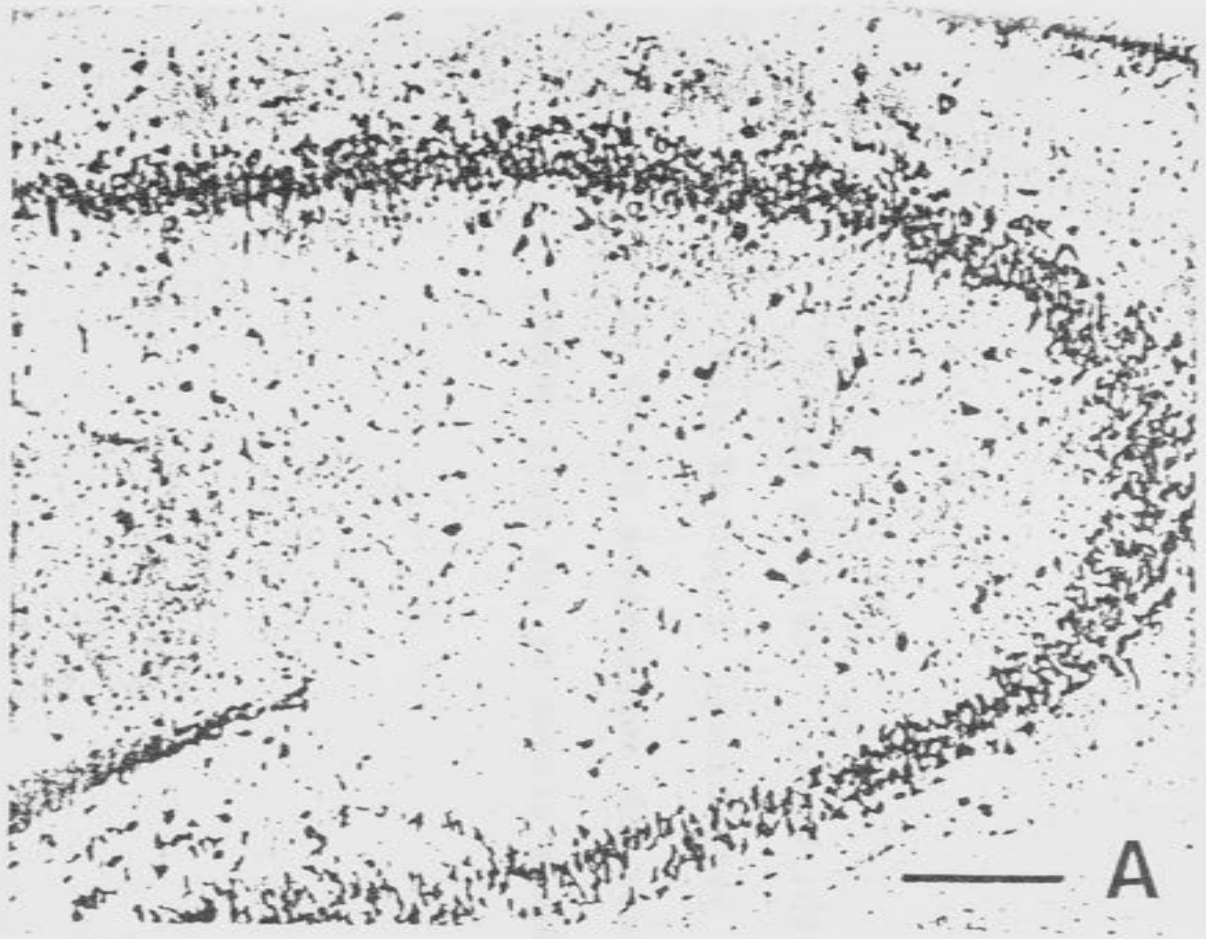

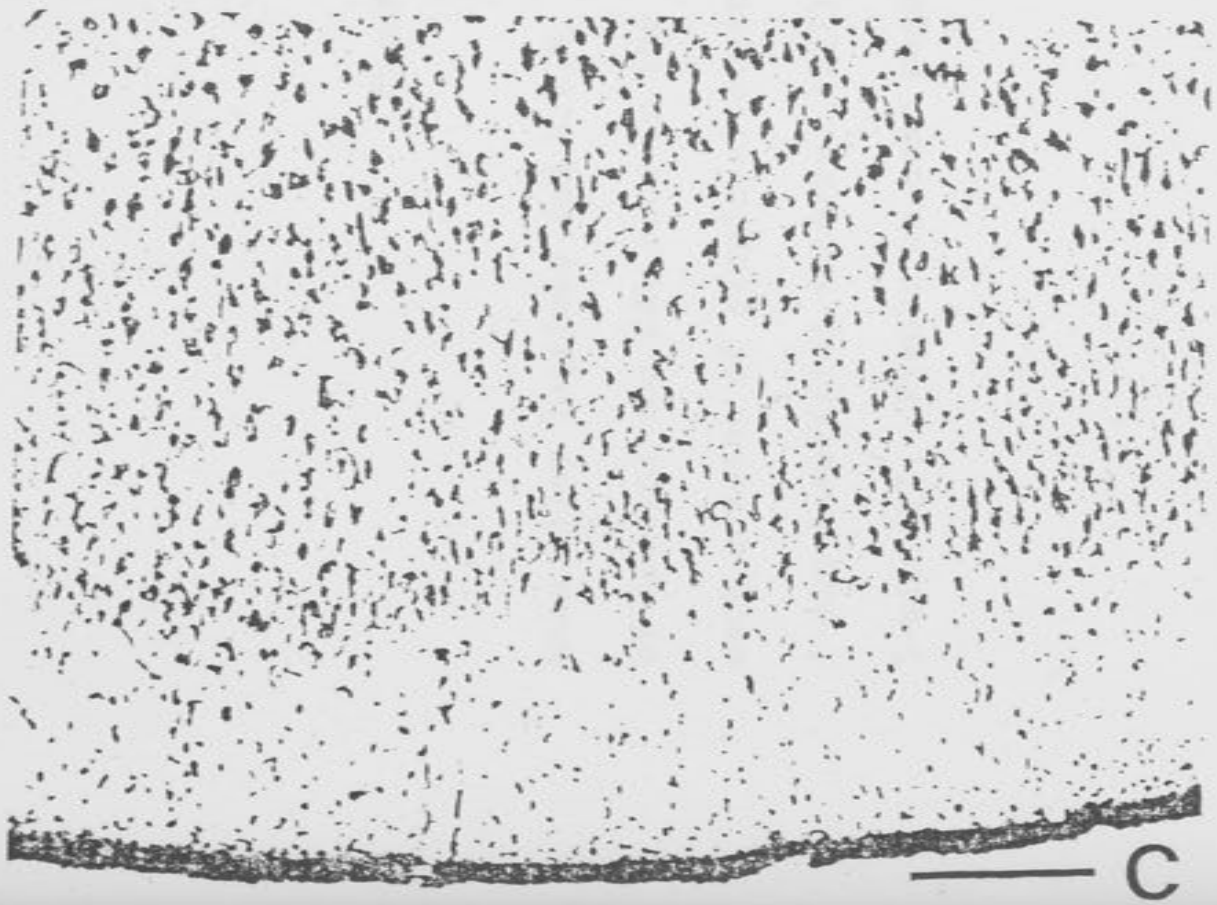

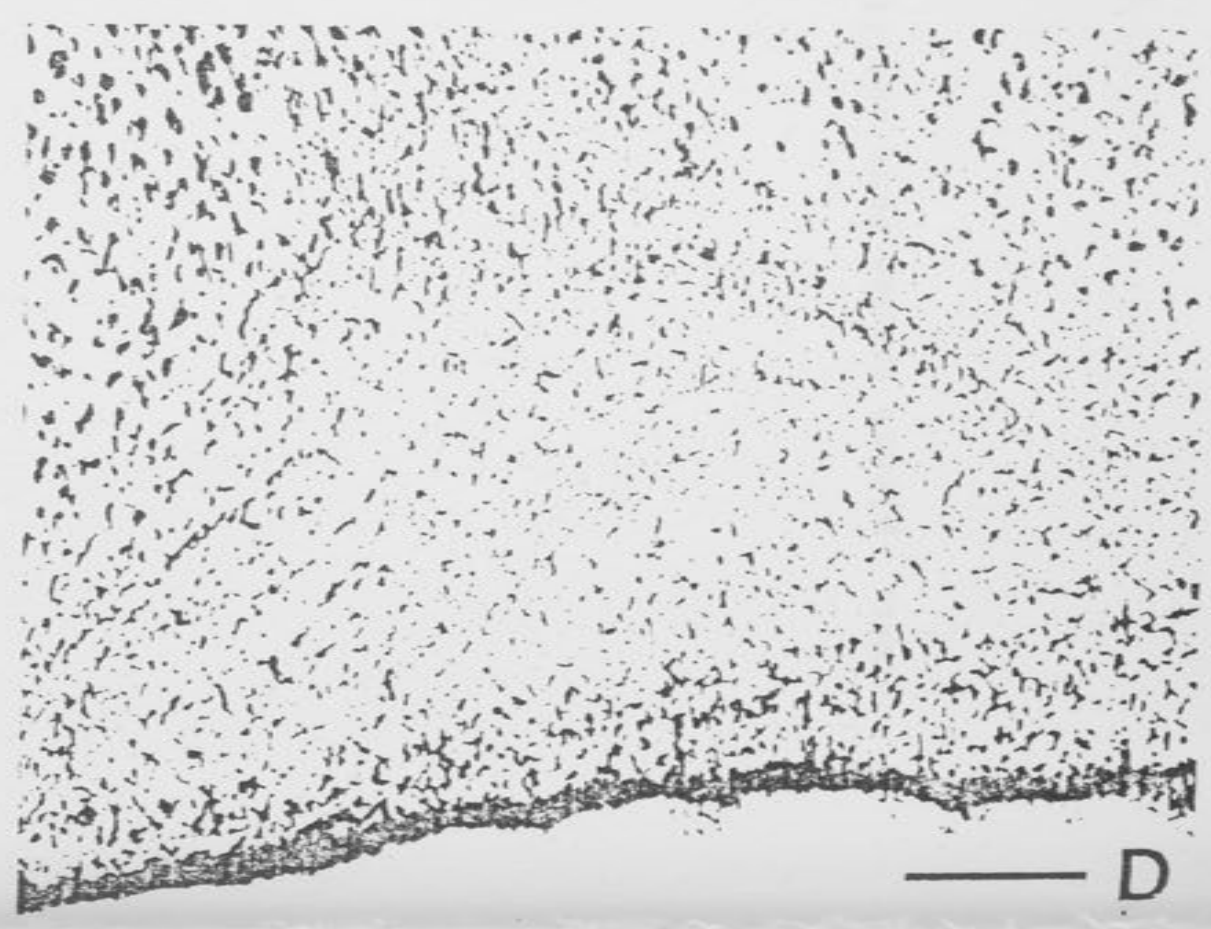

Figure 3 (Cont.)

Figure 4

Mean alley logtimes and S.E.M. (standard errors of the cell means) of control and KAL rats in the rewarded and nonrewarded trials of each session of single alternation. Left panel: training with 1-min intertrial interval (ITI). Right panel: training with 5-min ITI. Figure on next page.

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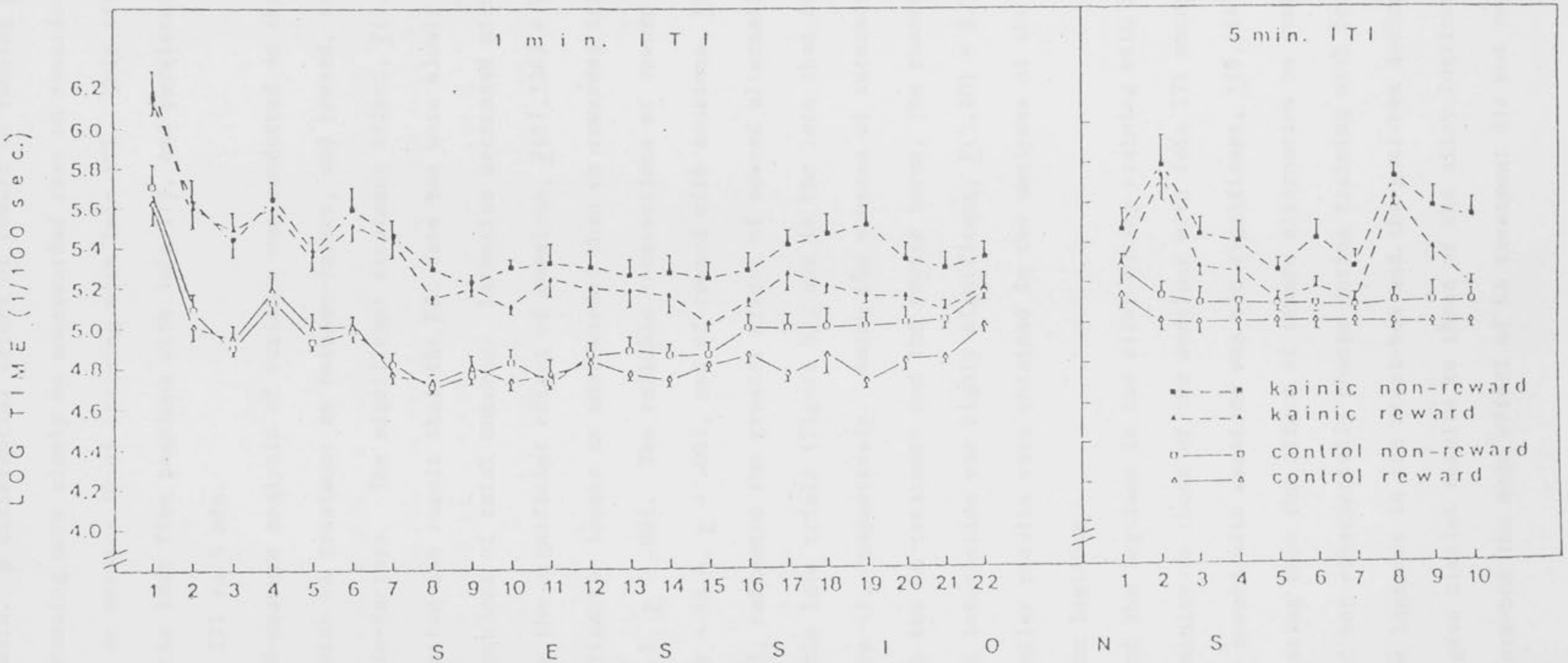

Figure 4 (Cont.)

alternating reward. A differential effect of reward on running performance, with the rats running more slowly on nonrewarded than on rewarded trials, first appeared on session 10 of training with 1min ITI. This effect stabilized in the last five sessions with 1min ITI, and persisted after increasing the ITI to 5 min.

A repeated-measure analysis of variance was conducted on the logtimes in the alley, with KA treatment as between-factor, and reward, sessions and trials as within-factors. The significant treatment effect, $F(1, 16) = 15.0$, $p < .001$, reflected the result that the KAL rats ran more slowly than the controls, independent of trial outcome. Latencies decreased with training, as indicated by the significant effect of sessions, $F(21,336) = 9.7$ $p < .001$, and were significantly longer on nonrewarded than on rewarded trials, $F(21,336) = 29.5$, $p < .001$. The reliable interactions of reward with trials, $F(5,80) = 46.4$, $p < .001$, and of reward with sessions, $F(21,336) = 4.2$, $p < .001$, reflected the greater effect of reward alternation in the early than in the late trials (Figure 5), and in the late than in the early sessions (Figure 4), respectively. Among the sources of interaction containing both the KA treatment and the reward terms, the treatment x reward x trials interaction was highly significant, $F(5,80) = 8.9$, $p < .001$. Essentially similar results were obtained in the analyses of the start-box and the goal-box logtimes.

Analyses of the logtimes in the alley during training with 5min ITI gave similar results to those of the sessions with 1min ITI except that the interaction of reward with sessions was not significant, $F(9,144) = 1.7$, $p > 0.1$, indicating that the effect of reward alternation on running performance did not substantially change during training with 5min ITI. Analyses of the logtimes in the start-box and the goal-box during training with 5min ITI gave similar results to those of the alley logtimes, except that in the start-box the main effect of KA treatment did not reach

Figure 5

Mean logtimes and S.E.M. of control and KAL rats on each rewarded or non rewarded trial in the blocks of initial five sessions with 1-min ITI (left panels), last five sessions with 1-min ITI (central panels), and last five sessions with 5-min ITI (right panels). Figure on next page.

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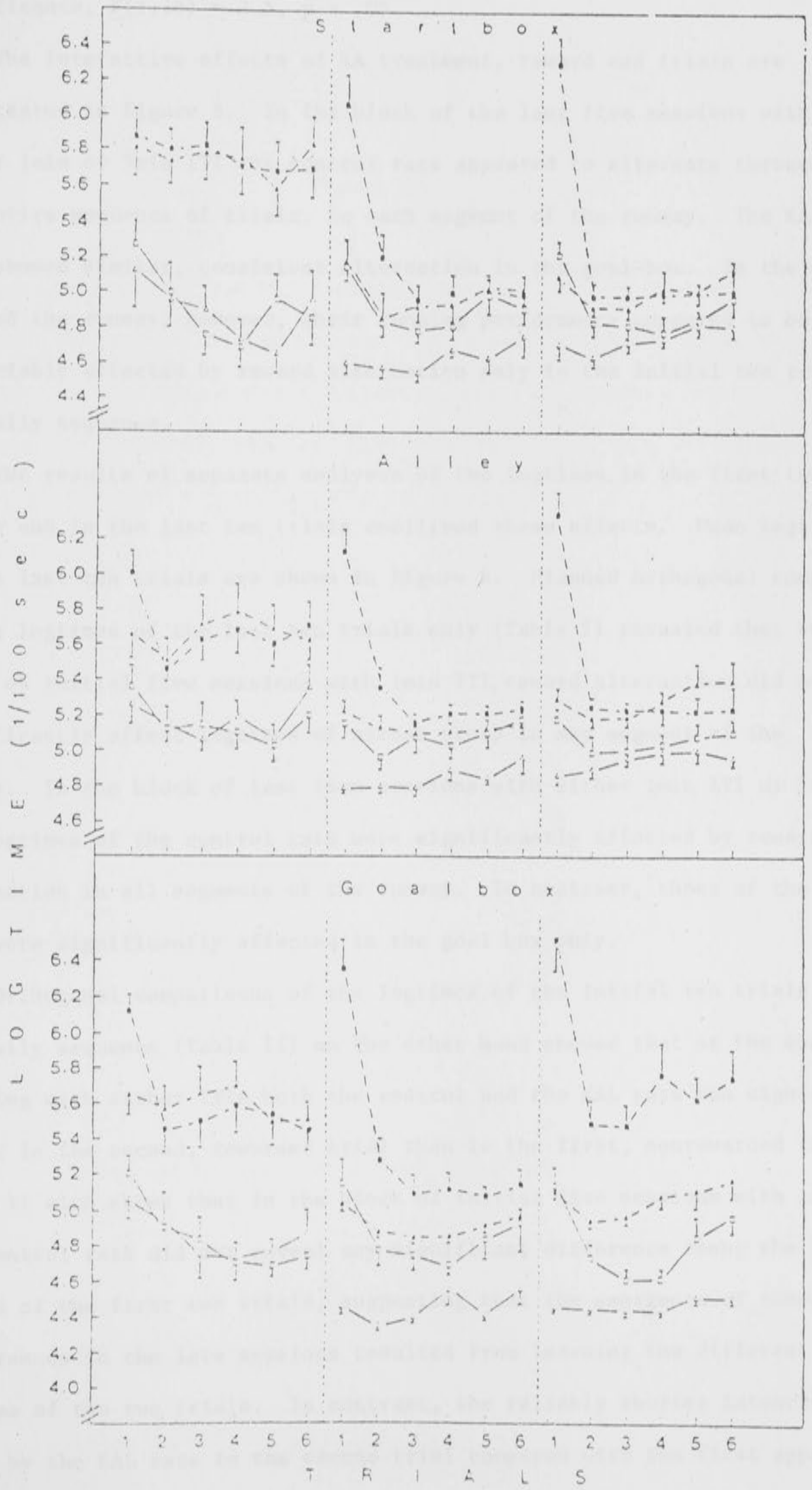
Starbox
Alley
Goalbox
LOG TIME (1/100 sec.)
TRIALS
6.4
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5.0
4.8
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4.2
4.0
1 2 3 4 5 6

significance, $F(1,16) = 3.5$, $p > .05$.

The interactive effects of KA treatment, reward and trials are illustrated in Figure 5. In the block of the last five sessions with either 1min or 5min ITI the control rats appeared to alternate throughout the entire sequence of trials, in each segment of the runway. The KAL rats showed similar, consistent alternation in the goal-box. In the start-box and the runway, however, their running performance appeared to be appreciably affected by reward alternation only in the initial two trials of the daily sequence.

The results of separate analyses of the logtimes in the first two trials and in the last ten trials confirmed these effects. Mean logtimes in the last ten trials are shown in Figure 6. Planned orthogonal comparisons of the logtimes of the last ten trials only (Table 1) revealed that in the block of initial five sessions with 1min ITI, reward alternation did not significantly affect logtimes of either group in any segment of the runway. In the block of last five sessions with either 1min ITI or 5min ITI the logtimes of the control rats were significantly affected by reward alternation in all segments of the runway. In contrast, those of the KAL rats were significantly affected in the goal box only.

Orthogonal comparisons of the logtimes of the initial two trials of the daily sequence (Table II) on the other hand showed that at the end of training with either ITIs, both the control and the KAL rats ran significantly faster in the second, rewarded trial than in the first, nonrewarded trial. Table II also shows that in the block of initial five sessions with 1min ITI the control rats did not reveal any significant difference among the latencies of the first two trials, suggesting that the emergence of these differences in the late sessions resulted from learning the different outcome of the two trials. In contrast, the reliably shorter latencies shown by the KAL rats in the second trial compared with the first appeared

Figure 6

Mean logtimes and S.E.M. of control and KAL rats in the last five rewarded (R) or nonrewarded (NR) trials of blocks of initial five sessions with 1-min ITI (left), last five sessions with 1-min ITI (center), and last five sessions with 5-min ITI (right). * = $p < .05$; ** = $p < .01$. Figure on next page.

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Figure 6 (Cont.)

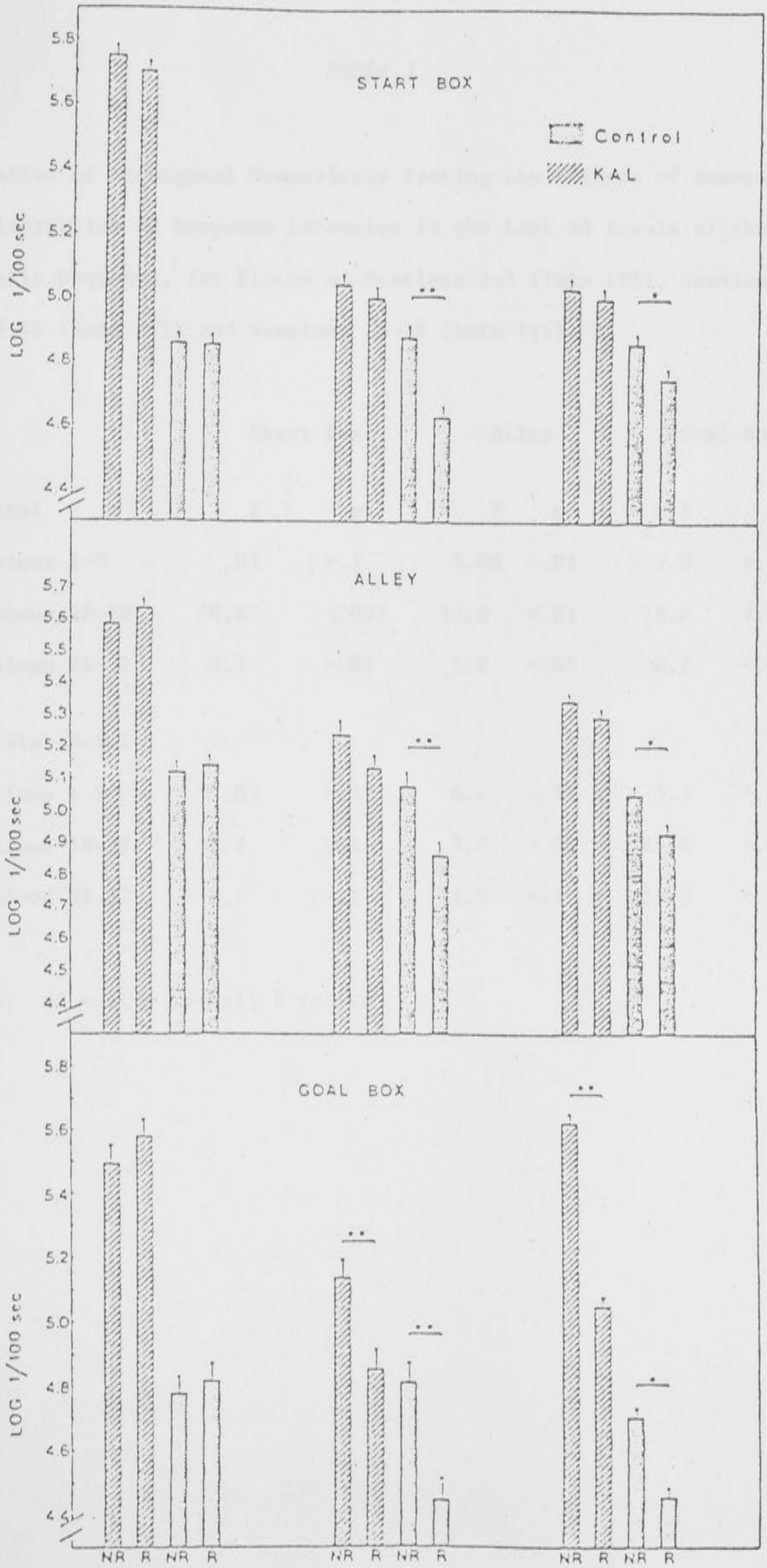

Table I

F Ratios of Orthogonal Comparisons Testing the Effects of Reward Alternation on Response Latencies in the Last 10 Trials of the Daily Sequence, for Blocks of Sessions 1-5 (1min ITI), Sessions 18-22 (1min ITI) and Sessions 28-32 (5min ITI).

	Start Box		Alley		Goal Box	
Control	F	p	F	p	F	p
Sessions 1-5	.01	>.1	2.88	>.05	1.0	>.1
Sessions 18-22	26.8	<.001	15.8	<.01	18.4	<.001
Sessions 28-32	6.5	<.05	5.2	<.05	4.7	<.05
Striatal Kainic						
Sessions 1-5	.03	>.1	0.4	>.1	3.3	>.05
Sessions 18-22	2.7	>.1	3.9	>.05	25.8	<.01
Sessions 28-32	0.8	>.1	1.9	>.1	25.8	<.001

Note: df = 1,16 for all F ratios.

Table II

F Ratios of Orthogonal Comparisons Testing the Effects of Reward Alternation on Response Latencies in the Initial Two Trials of the Daily Sequence, for Blocks of Sessions 1-5 (1min ITI), Sessions 18-22 (1min ITI) and Sessions 28-32 (5min ITI).

	Start Box		Alley		Goal Box	
Control	F	p	F	p	F	p
Sessions 1-5	1.7	>.1	1.3	>.1	0.3	>.1
Sessions 18-22	26.7	<.01	12.9	<.01	38.2	<.01
Sessions 28-32	29.2	<.01	19.9	<.01	43.6	<.01
Striatal Kainic						
Sessions 1-5	0.1	>.1	15.2	<.01	30.8	<.01
Sessions 18-22	61.6	<.01	86.9	<.01	109.6	<.01
Sessions 28-32	164.7	<.01	213.2	<.01	151.1	<.01

Note: df = 1,16 for all F ratios.

to result, in part, from warm-up effects, because these rats showed similarly reliable differences early in training, both in the alley and in the goal-box. Nonetheless, the performance of the KAL rats in these two trials also appeared to be affected by learning of their different outcomes: first, their start-box latencies in the initial two trials significantly differ from each other in the late but not in the early sessions (Table II). Furthermore, with training their latencies progressively increased in the first trial and decreased in the second, in all three segments of the runway (Figure 5).

Figure 7 shows the mean running performance during extinction. A repeated measure analysis of variance, with KA treatment as between-factor, and sessions and trials as within-factors, showed that neither the treatment main effect nor any of the sources of interaction containing the treatment term reached significance in either segments of the runway, ps > .05. However, subsequent multiple comparisons using the Neuman Keuls procedure (37) revealed that the logtimes of the KAL rats in the start-box, the alley and the goal-box were significantly longer than those of the controls in sessions 4 and 5, in session 5 and in sessions 1 and 2, respectively, ps < .05.

DISCUSSION

The control rats reliably alternated speed with ITIs up to 5min and they were able to do so both in the alley and in the start-box, i.e. in places remote from the food cup, suggesting that their alternation performance was not controlled by either food smell or after-effects of taste and other visceral stimuli. It thus appears that the effective source of stimulus control for the control rats was memory of the specific reward conditions prevailing in the previous trial.

In the last ten trials of the daily sessions the KAL rats alternated

Figure 7

Mean logtimes and S.E.M. in the last two sessions of food-reinforced alternation (pre-ext.) and in each sessions of extinction in the start box (left panel), alley (center panel) and goal box (right panel). Figure on next page.

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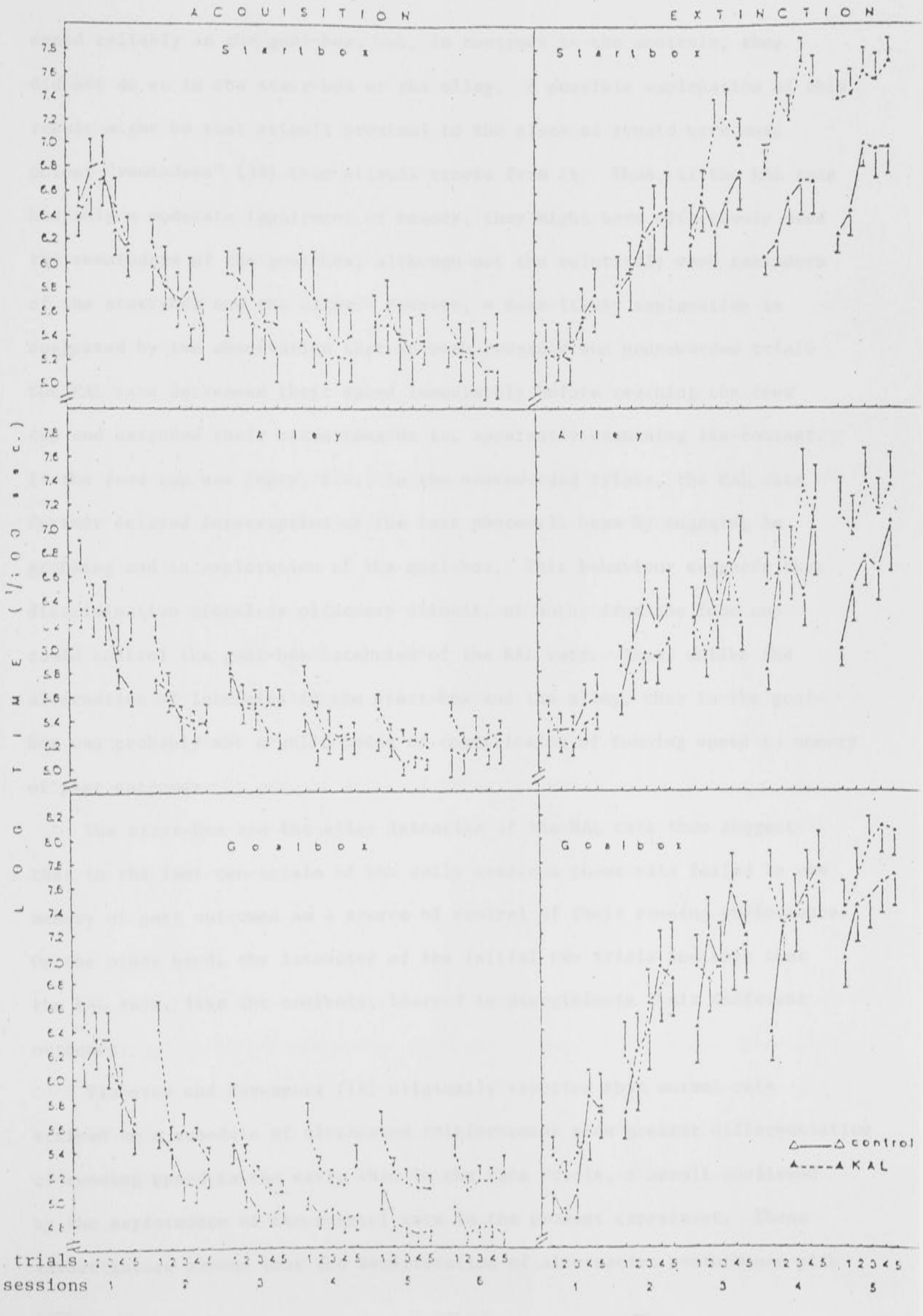
Figure 7 (Cont.)



speed reliably in the goal-box, but, in contrast to the controls, they did not do so in the start-box or the alley. A possible explanation of this result might be that stimuli proximal to the place of reward were more potent "reminders" (38) than stimuli remote from it. Thus, if the KAL rats had only a moderate impairment of memory, they might have effectively used the remainders of the goal-box, although not the relatively weak reminders of the start-box and the alley. However, a more likely explanation is suggested by the observation that on both rewarded and nonrewarded trials the KAL rats decreased their speed immediately before reaching the food cup and extended their heads towards it, apparently examining its content. If the food cup was empty, i.e., in the nonrewarded trials, the KAL rats further delayed interruption of the last photocell beam by engaging in grooming and in exploration of the goal-box. This behaviour suggests that discriminative visual or olfactory stimuli, or both, from the food cup could control the goal-box latencies of the KAL rats. Thus, unlike the alternation of latencies in the start-box and the alley, that in the goal-box was probably not a valid index of conditioning of running speed to memory of past outcomes.

The start-box and the alley latencies of the KAL rats thus suggest that in the last ten trials of the daily sessions these rats failed to use memory of past outcomes as a source of control of their running performance. On the other hand, the latencies of the initial two trials indicate that the KAL rats, like the controls, learned to discriminate their different outcomes.

Flaherty and Davenport (14) originally reported that normal rats trained on a schedule of alternated reinforcement show greater differentiation of running speed in the early than in the late trials, a result confirmed by the performance of the control rats in the present experiment. These investigators showed that the deterioration of alternation performance with

trials could be fitted to a model of stimulus distinctiveness originally proposed by Murdock (27) to account for the serial position effects found in human serial memory. They thus proposed a mechanism of proactive interference from accumulated experience to account for their findings. An increased susceptibility to interference-generating factors, with resulting failure to discriminate temporally unique events --e.g. which specific outcome occurred in the nth trial -- could thus adequately account for the impaired alternation performance of the KAL rats in the late trials. On the other hand, the result that the reward conditions prevailing in the initial two trials reliably affected the running speed of the KAL rats appears to suggest that the KA treatment did not interfere with learning of especially distinctive events, such as habitual nonreward at the onset of the sessions.

In addition to being impaired in conditioned speed alternation, the KAL rats ran reliably more slowly than the controls, independently of trial outcome. Hruska and Silbergeld (18) found that during spontaneous locomotion KAL rats take longer than controls to swing their limbs while striding. This locomotor impairment may, at least in part, account for the failure of KAL rats to attain relatively high speeds during both spontaneous walking (18) and conditioned running.

EXPERIMENT 2

In Experiment 1 the KAL rats did not show increased resistance to extinction of partially reinforced running. In previous studies, however, rats with coagulative destruction (34) or KA-induced degeneration (33) of the striatum revealed increased resistance to extinction of free-operant bar pressing after training on a schedule of continuous reinforcement (CRF).

Among the factors possibly accounting for these different results are differences of schedule of reinforcement during acquisition and differences

of temporal spacing of trials during extinction. In experiment 2 the rats were trained in the runway with a similar CRF schedule to that used in the bar pressing tasks (33, 34). Furthermore, to assess the role of temporal spacing of nonreward experiences on extinction rate, different groups of rats were trained with either massed or spaced trials.

Procedures

Male Wistar rats (Woodlyn Farms, Guelph, Ontario) weighing 275 - 300gm at the time of surgery were randomly assigned to a control group (N = 20) or a KAL group (N = 20). Surgery, postoperative recovery, food deprivation schedule, and adaptation to the runway were identical to those described in Experiment 1. The rats were trained to run on a CRF schedule, with five pellets as reward, for six daily sessions of five trials each. During acquisition training, the ITI was 10sec in the odd-numbered sessions and 10min in the even-numbered sessions for half the rats in each surgical group and vice-versa for the other half. The rats spent the ITIs in waiting boxes. On training subject with a 10sec ITI, control rats alternated with KAL rats, each rat running all its trials before the next rat commenced theirs. On training subjects with a 10min ITI, squads of five rats were used, including both control and KAL rats, with all rats of a squad running a given trial before the first rat of that squad commenced the next trial.

Following the last session of acquisition, the rats of each surgical group were paired on the basis of their mean alley latencies in the last two days of acquisition, with the two fastest animals in each group forming one pair, the slowest animals forming another pair and so on. A randomly selected rat of each pair underwent extinction with a 10sec ITI, and the other member of the pair underwent extinction with a 10min ITI. Extinction consisted of five daily sessions of five trials each, with no food ever available in the food cup.

RESULTS

Anatomical

Figure 8 illustrates the frontal plane with the largest area of neostriatal neuronal loss for each KAL rat. The neostriatal lesions were typically ovoidal, with center at A 7.8 - 8.2 and rostrocaudal extent ranging from 1.8 to 2.6mm. The rostral half of the globus pallidus showed bilateral, partial neuronal loss and astrocytic proliferation in nine KAL rats of the 10sec ITI group and eight KAL rats of the 10min ITI group. Damage outside the striatum included: 1) slight neuronal loss and astrocytic infiltration in the juxtacallosal layers of the frontal cortex above the injection site in seven KAL rats of the 10sec ITI group and in eight KAL rats of the 10min ITI group; 2) unilateral or bilateral degenerative changes and partial neuronal loss of the pyramidal neurons of either the CA1 or the CA3 hippocampal fields, or both, in nine KAL rats of the 10sec ITI group and eight KAL rats of the 10min ITI group; 3) bilateral, partial neuronal loss in the pyriform cortex of three KAL rats in the 10sec ITI group.

The brains of the control rats appeared to be intact except for a slight glial infiltration along the cannula tracks.

Behavioural

The running performance during acquisition is illustrated in Figure 9. The significant effects of trials, $F(5,180) = 17.0$, 38.7 and 52.3, $p < .001$, and sessions, $F(5,180) = 28.6$, 50.2 and 59.38, $p < .001$, on the start-box, alley and goal-box logtimes, respectively, reflected the increase of running speed with practice both within and across sessions. The main effect of KA treatment was significant in the goal box, $F(1,36) = 4.8$, $p < .03$, where the KAL rats ran more slowly than the controls, although not in the start-box or the alley, $Fs < 1$. The interaction of KA treatment with trials was significant both in the alley and in the goal-box, $F(4,864) = 3.7$ and 4.3, respectively,

Figure 8

Camera lucida drawings of frontal sessions showing the largest area of striatal neuronal loss in each KAL rat trained with either 10-sec ITI (left) or 10-min ITI (right) during extinction of continuously reinforced (CRF) running. Figure on next page.

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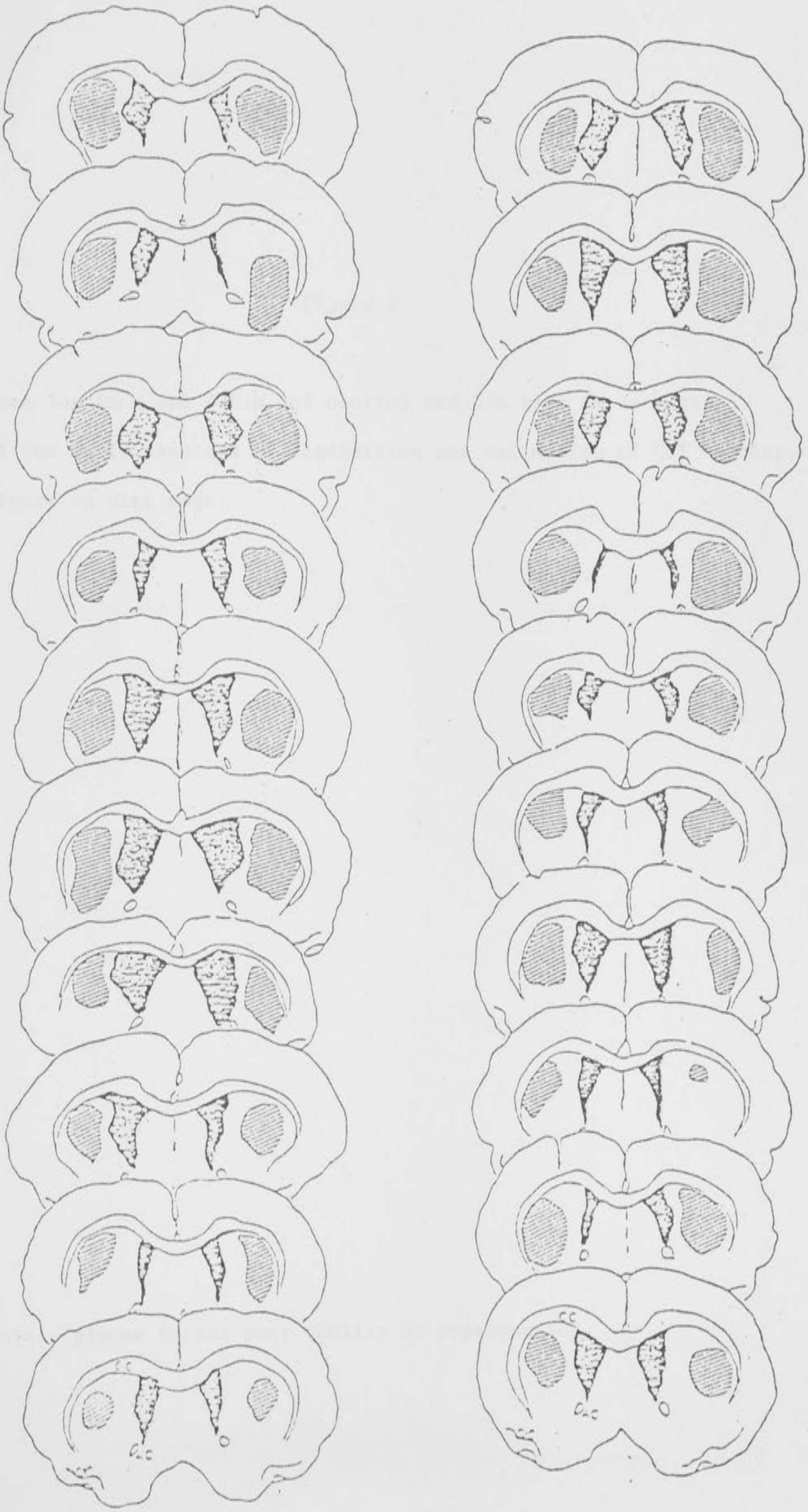

Figure 9

Mean logtimes and S.E.M. of control and KAL rats in each trial of the daily sessions of acquisition and extinction of CRF running. Figure on next page.

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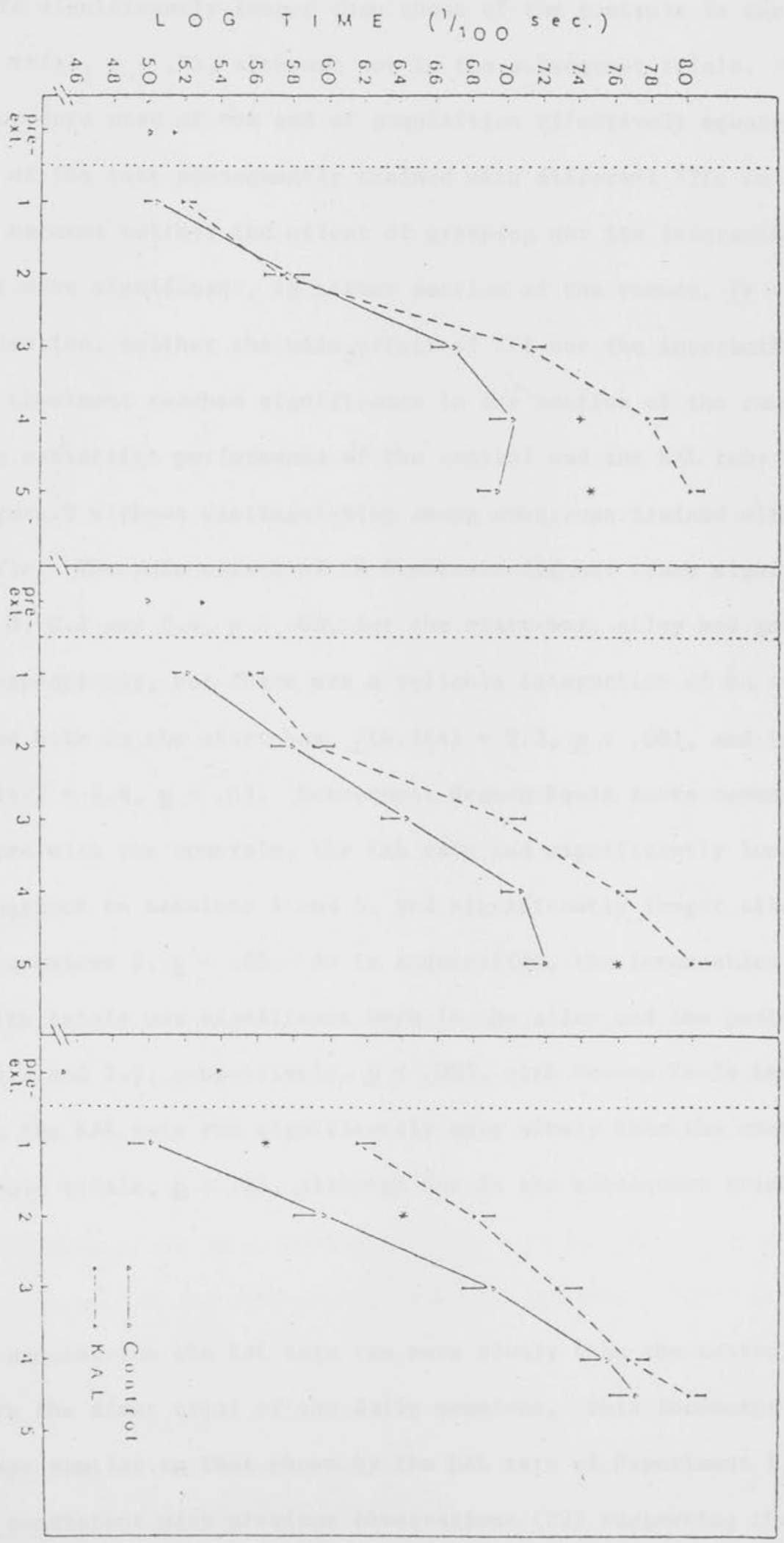
Figure 9 (Cont.)



$p < .01$, with Neuman-Keuls tests showing that the alley logtimes of the KAL rats were significantly longer than those of the controls in the first daily trial, $p < .05$, although not in the subsequent trials. The matching procedure used at the end of acquisition effectively equated the performance of the rats subsequently trained with different ITIs in extinction, because neither the effect of grouping nor its interaction with KA treatment were significant, in either section of the runway, Fs < 1.

On extinction, neither the main effect of ITI nor the interaction of ITI with KA treatment reached significance in any section of the runway, Fs < 1. The extinction performance of the control and the KAL rats is thus shown in Figure 9 without distinguishing among subgroups trained with different ITIs. The main effect of KA treatment did not reach significance, $F(1,36) = 3.0$, 0.2 and 0.4, $p > .05$, for the start-box, alley and goal-box logtimes, respectively, but there was a reliable interaction of KA treatment with sessions both in the start-box, $F(4,144) = 5.3$, $p < .001$, and in the alley, $F(4,144) = 2.8$, $p < .03$. Subsequent Neuman-Keuls tests revealed that, compared with the controls, the KAL rats had significantly longer start-box logtimes on sessions 4 and 5, and significantly longer alley logtimes on sessions 5, $p < .05$. As in acquisition, the interaction of KA treatment with trials was significant both in the alley and the goal-box, $F(4,720) = 5.3$ and 3.9, respectively, $p < .005$, with Neuman-Keuls tests showing that the KAL rats ran significantly more slowly than the controls in the first daily trials, $p < .05$, although not in the subsequent trials.

DISCUSSION

During acquisition the KAL rats ran more slowly than the controls, especially in the first trial of the daily sessions. This locomotor impairment was similar to that shown by the KAL rats of Experiment 1, and it was also consistent with previous observations (29) suggesting that KA-induced lesions of the striatum impair initiation of locomotor responses.

On the other hand, the KAL rats showed no appreciable alterations either in rate of learning of approach responding or in response suppression during extinction, independent of temporal spacing of the trials. In fact, consistent with the results of Experiment 1, the KAL rats showed an even greater suppression of approach responding than the controls in the late extinction sessions. Thus, despite the use of both a CRF schedule and massed trials, the KAL rats did not demonstrate a similar increase in resistance to extinction to that previously shown during extinction of free-operant bar pressing (33).

The present data thus agree both with those of Experiment 1 and with those of a recent study (12) showing that KA-induced striatal degeneration does not result in increased resistance to extinction of approach responding in a T maze. The increased rate of bar pressing and the decreased locomotor speed of KAL rats during extinction appear to be consistent with the interpretation (29) that the striatum has a facilitatory role on locomotor activity and an inhibitory role on repetitious, manipulative behaviour.

GENERAL DISCUSSION

The main finding of this study was that neuronal loss induced by striatal injections of KA impaired conditioning of speed alternation. The neuronal loss did not prevent adequate learning of relatively simple associations, because it neither decreased rate of acquisition nor increased rate of extinction of either partially or continuously reinforced running. Therefore, the impairment of conditioned speed alternation cannot be attributed to an alteration of basic associative processes, such as a decreased sensitivity to either reward or nonreward.

A general amnesia for discrete events also does not seem to account for the impairment of alternation, because the KAL rats appeared to be able to discriminate among outcomes of the initial trials of the daily

alternation sequence, thus suggesting adequate memory of relatively distinctive events. The failure to alternate specifically in the late trials of the daily alternation sequence rather indicates an increased susceptibility to factors promoting proactive interference, with resulting selective impairment of serial memory performance.

The precise mechanism of this apparent memonic alteration remains to be elucidated, however. A direct damage to temporal information processing might be involved. However, other kinds of alterations might have indirectly affected serial memory performance. Thus, an abnormally high response-invigorating effect of reward might have impaired conditioned speed alternation by competing, in the nonrewarded trials, with the response-decelerating effects of memory of recent reward.

An increased sensitivity to highly arousing stimuli has indeed been proposed to account, at least in part, for the impairments of rats with coagulative lesions of the striatum (19), rats with KA-induced neuronal loss in the striatum (29) and patients with HD (4) in tasks of learning and memory. Specifically, we previously found that KAL rats are impaired in food-reinforced spatial alternation but not in spontaneous alternation (29). Performance of spontaneous alternation did not involve a primary reinforcer and was measured on the basis of fewer daily trials than food-reinforced alternation. It was therefore suggested that either an excessive arousal reaction to appetitive stimuli or an increased susceptibility to interference resulting from accumulated trials, or both, more adequately accounted for the failure in food-reinforced spatial alternation than the alternative interpretations of a general failure of recent memory or a failure of spatial discrimination. The present findings, indicating a selective impairment of serial memory performance in a nonspatial task, appear to confirm and to add scope to that interpretation.

Besides impairing speed alternation performance, the KA-induced lesions

resulted in a decreased speed of locomotor responding, this effect being especially evident at the onset of the training sessions. This result is consistent with those of Hruska and Silbergeld (18) and complements that of a previous study (29) in which KAL rats showed less locomotor activity than controls at the onset of an exploratory task. Taken together, these findings suggest that, in addition to the alterations of stride demonstrated by Hruska and Silbergeld (18), an impairment of mechanisms facilitating initial engagement in locomotor activity might account for the motor alterations of the KAL rats in the present study. It is of interest that a similar reluctance to initiate motor tasks has been described in HD patients (5), a finding that further supports the analogy between the KA striatal model and the human disease.

Early studies of the neurotoxic effects of KA injections in the neostriatum did not report KA-induced extrastriatal neuronal loss (22,35). However, this phenomenon has been more recently demonstrated by several investigators (9,40) and confirmed in our laboratory (29) while this study was in progress. The present histological results agree with those of these investigations by showing various degrees of neuronal loss in the hippocampus, pyriform cortex, globus pallidus and frontal cortex of several KAL rats.

The failure of KA neostriatal injections to confine their neurodegenerative effects to the neostriatum does not detract from the usefulness of the KAL rat preparation as a model of HD, because this disease, at least in its advanced stages, results in neuronal loss in both the caudate-putamen and extrastriatal structures such as the neocortex, hippocampus and globus pallidus (3,15,24,31). Indeed, Coyle and his collaborators, originally failing to detect extrastriatal damage after KA striatal injections, suggested additional injections of KA to the globus pallidus and the cortex to obtain a more faithful model of the pathologic sequelae of HD (11).

On the other hand, the multifocality of the KA-induced lesions clearly complicates the anatomical interpretation of the behavioural results. Thus, although the greater severity, symmetry and reliability of occurrence of striatal neuronal loss compared with that in extrastriatal structures might be taken to suggest a main role of striatal damage in the observed behavioural impairments, a contribution of the extrastriatal pathology cannot be presently excluded.

Although diffusion of KA from the striatal injection site might account for the neuronal loss in close structures such as frontal cortex and globus pallidus, the mechanism of neuronal loss in distant areas such as the hippocampus and the pyriform cortex remains to be fully elucidated. Recent studies have shown that the hippocampal neuronal loss induced by either amygdalar (1) or systemic (16) injections of KA can be prevented by pretreatment with antiepileptic drugs such as diazepam, suggesting a causal relation between KA-induced, postinjection seizures and hippocampal pathology. A similar mechanism might account for the hippocampal neuronal loss resulting from KA striatal injections, because these injections also induce repeated episodes of seizures postoperatively (29,40). It is thus possible that the combined use of antiepileptic drugs and KA striatal injections might induce neuronal loss well localized to the neostriatum, thus preventing ambiguities in the anatomical interpretation of the behavioural sequelae. Further studies are warranged to examine the viability of this method.

ACKNOWLEDGEMENTS

The excellent technical assistance of Stella Atmadja and Lisa Gaelick is appreciated. M. Pisa is an MRC Fellow.

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MEASURING FEEDING RESPONSES IN OPERANT RESEARCH

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The numbers of reinforcements and responses are the only dependent measures recorded by most of the commercially available self-contained operant conditioning consoles, e.g., Lafayette Model 81320 and BRS/LVE Model 3000. Perhaps just as important is an indication of whether an animal has actually consumed the food reward. One advantage of such a dependent measure could be seen, for example, in studies where animals show a resistance to extinction following various brain lesions (3, 6). If during the extinction schedule the animals, although responding at higher rates than controls, did not attempt to consume the food reward, this would be indicative that a learning or memory impairment was not the sole contributor to the extinction deficit. In fact, such a finding in decorticate rats has been noted (4): while decorticate rats would keep responding during the NOGO (extinction) component of a GO-NOGO schedule they would not attempt to get any food when none was available. Thus the animals must have learned the non-reward schedule.

The advantages of adapting a standard pigeon grain-feeder for use in rodent as well as avian operant research has been described (2). It reduced costs thirtyfold, and stockpiling or hoarding of food was virtually eliminated. This allows a more precise control over the presentation and accessibility of food than possible in commercially available food pellet dispensers. We have further modified a standard pigeon grain feeder (BRS/LVE Model GEM-001) to record feeding responses. All that has to be done is to place a photocell beam across the food cubicle so that interruption of the beam occurs when the animal's head enters. Beam interruptions are counted as feeding responses on an incremental counter. Placement of the beam 1½ cm inside the intelligence panel and 1 cm above the lower edge of the feeder entry hole gives a regression line slope of about 1.0 and *rs* of .80 to .99 between actual head entries and beam interruption counts. This falls short of a measure of ingestion on each trial but if consumption might differ from feeding responses, this could be estimated over a testing session by weighing the contents of the hopper. For those who use self-contained operant programmers, more photocell detectors (1) and counters (5) can be built quite easily and inexpensively. Our use has been successful.

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Accepted October 12, 1979.

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Physiology & Behavior, Vol. 23, pp. 795–797. Pergamon Press and Brain Research Publ., 1979. Printed in the U.S.A.

Digital Counters: Inexpensive Alternatives

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Received 4 May 1979

SANBERG, P. R. AND W. P. BELLINGHAM. *Digital counters: Inexpensive alternatives.* PHYSIOL. BEHAV. 23(4) 795–797, 1979.—Two rapid methods for converting inexpensive calculators into counters for use with digital logic controlling systems are described. The first method utilizes integrated circuit opto-couplers with built in switching transistors, and the second uses inexpensive CMOS solid state switches. Using one of these techniques almost any calculator can be converted, and operated directly from digital logic without the use of interface hardware, such as DC drivers.

Counters Calculators Digital logic Behavioral equipment

COUNTERS are an essential part of a behavioral laboratory, and with the ever increasing number of dependent measures needing recording, it is not uncommon for an average size operant laboratory to have a multitude of counters. However, the purchasing of a quantity of counters from behavioral equipment manufacturers can become enormously costly; about $500 for a rack of ten counters. Furthermore, the counters most commonly distributed by these companies are electro-mechanical therefore having inherent problems of speed limitation, noise and relatively poor reliability. Thus, the need for technologically more advanced and economical counters becomes apparent.

Alexandrovich [1] first demonstrated a simple way in which an inexpensive electronic calculator with LED display can be converted to a solid state counter by simply making contact closures on either the "+" or "=" key (depending on the add mode of the calculator) after the calculator has been placed in a constant addition mode. Applying this idea to behavioral research, Wolach, Roccaforte and Breuning [6] demonstrated a conversion technique for calculators that would allow interfacing with control equipment. Their technique essentially consisted of entering counting signals to the calculator via a relay which substituted its contact closure for one produced by manually depressing the "+" or "=" keys. In the sense that relays provide total electric isolation of each counter from another and from the control apparatus, the solution is ideal. However, relays are mechanical and therefore relatively unreliable as well as slow and noisy. In those increasing cases where control is by some form of solid state circuitry the use of relays is particularly disadvantageous. Drivers must be used to activate the relays which, in turn, activate the counters. Thus, reliability goes down while space requirements and cost go up. A solution to these problems was provided by Rayfield [5] in which a transistor-resistor circuit was used to make the necessary contact closure. Several advantages accrue from this solution. The DC driver is eliminated since digital logic modules can directly activate the transistor due to its low power demands. Transistors are quiet, cheap and fast. A potential problem with this method, however, is that it does not provide complete electrical isolation between the counters and the control logic. Opto-couplers, such as the 4N26, can solve this problem. An opto coupler is a small integrated circuit with a built in light emitting diode (LED)-photoresistor circuit which activates a switching transistor (see [3], cost approx. $1.00). Thus the incoming logic current only lights the LED and is therefore completely isolated from the transistor (see Fig. 1).

Figure 1 illustrates the conversion of a Royal Model 91S calculator into a counter using a 4N26. The Royal calculators we have used, Models 90S and 91S, are simple to modify since the keyboard wire terminals are pre-numbered. The two keyboard wires (equivalent to the "=" key) for the Royal 90S and 91S are keyboard terminals 5 and 7, and 6 and 15, respectively. These are wired to the emitter pin (pin 4) and collector (pin 5), respectively, of the transistor in the 4N26. Keyboard terminal 1 of both calculators which is the power supply ground of each calculator is wired to the cathode (pin 2) of the LED in the 4N26. The LED anode (pin 1) is connected to the incoming count pulses. Pins 3 and 6 are not connected. A low to high logic count pulse will momentarily light the LED and switch the transistor, thereby adding a 1 count to the running total of the calculator.

We have found a difficulty with this method, however, in that not all calculators have a sufficient bias on the contact closure to switch a transistor. The National Semiconductor Model 850A is one example. In this latter case we found that the CMOS 4066 quad-bilateral single pole–single throw switch worked quite well. This particular device can operate on supply voltages from +3 VDC to +15 VDC, has high noise immunity, low "ON" resistance and very low power requirements. The 4066 has four switches incorporated in it with low crosstalk between the switches. Isolation between the control pulse and the counter exists. Therefore the 4066 will work on those calculators in which an opto-coupler is inappropriate. The 4066 can be purchased for a modest $1.00 or less. The CMOS 4016 is a similar device that is pin for pin compatible with the 4066, but has a higher "ON" resistance. It should work equally well.

Figure 2 is a detailed description for the conversion of the

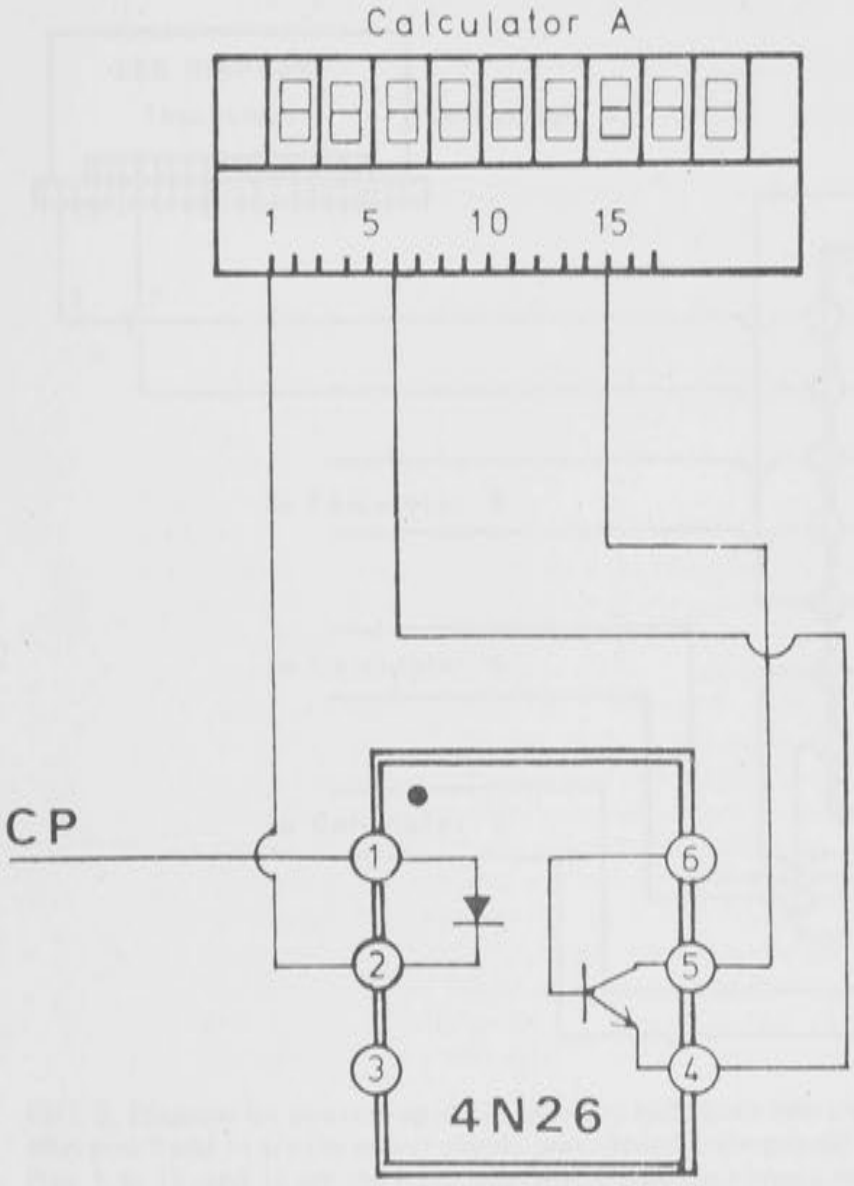

FIG. 1. Diagram for converting an inexpensive calculator into a digital counter using a 4N26 opto-coupler. On the 4N26 pins 1 and 2 are the anode and cathode, respectively, of the LED. The anode should be connected to the incoming count pulse (CP) (see [3]). Pins 4 and 5 are the emitter and collector, respectively, of the switching transistor and should be connected to the keyboard input wires of the "=" or "+" key of the appropriate calculator (see [6]). Wires for the "=" or "+" key on any calculator can be found by shorting two keyboard input wires at a time until the calculator performs an addition, after being placed in the constant add mode (see [6]). The example calculator (calculator A) shown in the diagram is a Royal Model 91S.

National Semiconductor Model 850A using the CMOS 4066. We found two different internal structures in the National Semiconductor 850A calculators we obtained from the same supplier. The first type had the calculator integrated circuit directly attached to the keyboard and a row of flexible wires from the keyboard to the LED display. The second type had the calculator integrated circuit detached from the keyboard and connected by a row of flexible wires. The two wires corresponding to the "=" key in these calculators are in the set of flexible wires aforementioned for each type of calculator. For the first type, from the side opposite the external power supply, wires 2 and 7 are the necessary lines. For the second type, wires 3 and 8 are the correct lines. After the calculator has been placed in the constant addition mode, a contact closure between the two appropriate lines for each calculator will cause an additional count to the running total.

Wires were soldered to the appropriate lines and connected to the appropriate pins on the 4066, as illustrated in Fig. 2. Also illustrated in Fig. 2 are the pins corresponding to the digital logic input for each switch and the power for the CMOS unit. The switch is open when the digital input is low and closed when it is high. Thus, a low to high input pulse will close the switch momentarily, causing a count. It should be noted that when power to the 4066 is off, the switches are closed. This is equivalent to keeping the "=" key depressed.

Rayfield [5] suggested that by having switch closures on other keys, up-down and automatic resetting counters could easily be made. The 4066 would be quite advantageous for these kinds of applications since each small package contains four switches.

The calculators we have used, National Semiconductor 850A and Royals 90S and 91S, differ dramatically in the structure of the internal components, but are easily modifiable using one of the methods described. The principles are the same for most calculators and with care they should be easily convertible. Reference to Wolach *et al.* [6] will prove valuable. The major problem that will be encountered with other types of calculators will be determining which lines control the addition function, but the principle is simple. All one needs to do is open the calculator and systematically short two keyboard wires at a time on the keyboard output strip until the calculator performs an addition. The other difficulty that may present itself is gaining access to the wires from the keyboard itself. Wolach *et al.* discuss this problem and note which calculators are easily modifiable.

For those desiring to avoid the expense of constantly replacing the calculator's batteries Rayfield [5] suggested using an appropriate integrated voltage regulator. This is an excellent and inexpensive method but we would like to advise the builder to consult the technical information regarding the voltage regulator employed. It is not clear from Rayfield's article that proper capacitance needs to be supplied and failure to do so could easily damage the circuit. Reference to handbooks such as the *Voltage Regulator Handbook* [4] and *Cmos Cookbook* [2] should prove quite useful.

It is evident from the above discussion that calculator counters offer more advantages than just cost to the behavioral scientist. These include more digits, more visible display (LED, LCD or fluorescent type), speed, better reliability, and the virtual absence of interfacing hardware, such as DC drivers and mechanical relays. Even the minor problem reported by previous investigators [6] that the value 1 must always be subtracted from your final result, because the counter starts with 1 instead of 0, is easily overcome. In our case, we merely subtracted 1 prior to initiating the resetting sequence, as described by Wollach *et al.* ([6], i.e., "+" 1 "="), which then started the counter at 0.

In conclusion, a rack of ten counters was made in our laboratory and connected to the digital logic controlling system, in less time and at one-tenth the price of installing a rack of typical mechanical type counters.

ACKNOWLEDGEMENTS

We are indebted for the assistance and suggestions of R. Dalby, D. Crawford and R. Jackson.

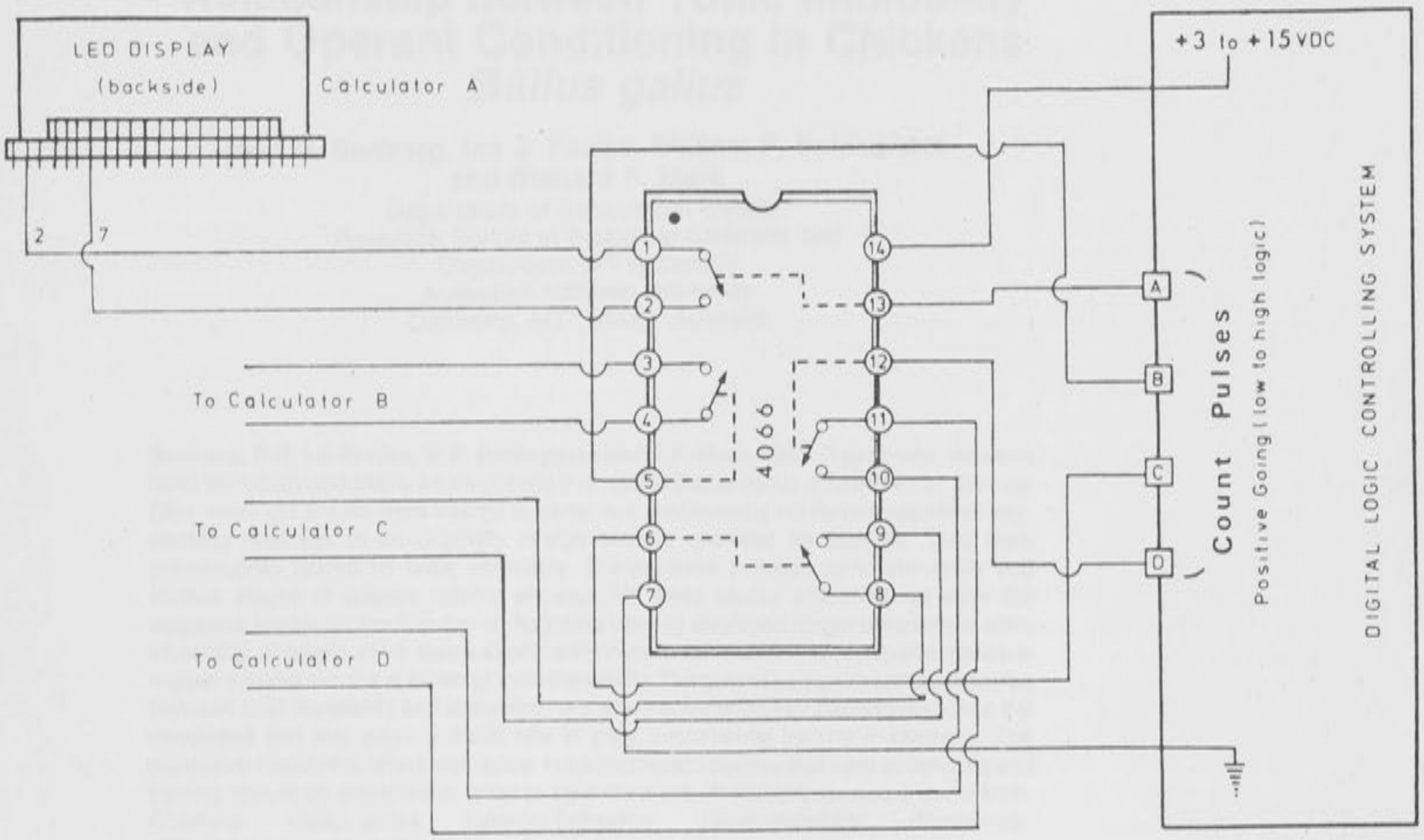

FIG. 2. Diagram for converting an inexpensive calculator into a digital counter using CMOS 4066 single pole-single throw switches. On the 4066 pins 7 and 14 are the power supply pins. Seven is the ground and 14 is the + VDC. Any logic system using +3 V to +15 VDC is feasible. Pins 5, 6, 12, and 13 are the input pins that create the closure on their respective outputs. When the 4066 is switched off there is a closed circuit on the output pins. The example calculator (calculator A) shown in the diagram is a National Semiconductor 850A.

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Bird Behaviour 3: xx-xx. *Short Communication* PRINTER'S PROOFS

Relationship between Tonic Immobility and Operant Conditioning in Chickens *Gallus gallus*

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Sanberg, P.R, I.J. Faulks, W.P. Bellingham and R.F. Mark. 1981. Relationship between tonic immobility and operant conditioning in chickens *Gallus gallus*. *Bird Behaviour* 3:xx-xx.
Four week old chicks were trained to perform a continuously reinforced appetitive key-pecking response in an originally design operant chamber for pigeons. They were subsequently tested for tonic immobility. Comparisons between tonic immobility and various stages of operant training showed that those chicks which did not enter the magazine feeder on the first day of magazine training displayed longer durations of tonic immobility. Similarly, there was a significantly inverse correlation between performance in hopper training and the duration of tonic immobility. There were no significant relationships between tonic immobility and acquisition or performance of the key-pecking response. It is concluded that fear plays a major role in early instrumental training in chickens. The excessive neophobia which can occur in some chicks requires that care in handling and training should be observed in order to train chickens adequately for operant research.
Chickens Gallus gallus Operant behaviour Tonic immobility Continuous reinforcement Handling

Introduction

As a subject of investigation, chickens have become quite popular in psychological and behavioural research. Most commonly young chickens have been used for studies elucidating mechanisms of learning (7, 9), memory (2) and affective states (3, 6). Experimental paradigms for measuring behaviour have included imprinting (1, 3), taste aversion (7), avoidance (2), simple discrimination (9), maze learning (5, 8) and tonic immobility (3, 6, 8). However, compared to other bird species, especially pigeons, chickens are rarely used in operant research. Young chicks can autoshape (14) and instrumentally key-peck for heat (15) or food reinforcement (4), but, few, if any, investigators have used chicks over one week old in these studies. One obvious reason is that old chickens are too large for the standard Skinner boxes for pigeons which are commercially available. Another likely reason was noticed in the process of evaluating the effects of forebrain injections of cycloheximide or glutamate on appetitive key-pecking in chickens older than three weeks (12). We observed in preliminary experiments that many control animals would not acquire key-pecking in response to a food reward when handled like other types of animals. Many chickens appeared to be neophobic to the new test environment. Procedures designed to minimize neophobia in subsequent studies resulted in successful acquisition of key-pecking in almost all chickens. Since these observations suggested that fear may influence operant responses, and because tonic immobility, a good measure of fear in chickens (6), increases after the first week of life (6), the present study was performed to compare directly the relationship between operant conditioning and tonic immobility in chickens.

Methods

Thirty male chickens which were crosses between White Leghorn and Black Australorp breeds were obtained from Poultry Research Farm (Victoria) on day one of life. They were kept in groups of four to six birds until day 14 when they were kept in pairs for the remainder of the study. Pairs were housed in metal cages, 23 x 23 x 29 cm, with a clear perspex front. Constant warmth (25°) and light were provided by overhead lamps (25W). Chicks had free access to crumble (Hutmil, Victoria) and water (tap water with teramycin added) during the study, except when under deprivation as described below.

Four standard BRS Foringer Skinner boxes for pigeons, enclosed in soundproof chambers, were used. Each was fitted with an illuminated pecking key located immediately above a grain feeder. The boxes were modified with a grid floor of adjustable height, with an enlarged grain hole and with a photocell beam across the food cubicle so that interruption of the beam occurred when the animal's head entered, as described elsewhere (10). Programming and stimulus delivery were controlled by Digital K-logic solid state, circuitry. Key-pecks were recorded on printing counters converted from Royal 310PD printing calculators. Photocell interruptions and reinforcements were recorded on counters converted from National Semiconductor 850A calculators (see 11). Chicken crumble was used as the reinforcer.

At three weeks of age the chickens were permitted only 1.5 h of feeding daily, between 9:00 and 11:00 pm. One week later the animals were first given two 20 min magizine training sessions on days one and two, respectively. The animals were very gently placed in the operant chamber, which had the grain feeder up and had extra grain in the food hopper (days one and two) and scattered on a paper towel on the grid floor immediately before the hopper entrance (day one). Handling consisted of perching the chicken on the index finger, and allowing it to step voluntarily into the operant chamber — the conventional method consists of grasping the bird about the body and manually placing it within the chamber. On days three to five, the animals were given hopper training over 20 min sessions. On days three and four the feeder (hopper) delivered grain to the food cubicle for 10 s followed by 5 s of non-delivery. Day five consisted of food delivery of 10 s followed by 20 s of non-delivery. Photocell interruptions were measured over days one to five. The animals were then given daily sessions of 30 min each, in which the hopper delivered grain for 5 s every 30 s. In each session the free grain delivery was preceded by 5 s of red illumination of the pecking key, which ceased when the grain was delivered (autoshape period). In addition, at anytime when the red illumination was not present, the bird received a reinforcement of 5 s of grain access (continuous reinforcement period), if the key was pecked. A printing counter recorded those pecks which occurred during either the autoshape or continuous reinforcement schedules over 30 s periods. When an animal key-pecked on any one session during the continuous reinforcement period, on the next day it was given one more continuous reinforcement session only. This was 30 min long, the pecking key was not illuminated, and only crumble (5 s access) contingent on key-pecking was available.

All animals successfully acquired the continuously reinforced key-peck response by day 10. Starting on day 13 the animals were tested for tonic immobility. Tonic immobility was tested after the appetitive key-pecking experiment, because Nash and Gallup (8) have demonstrated that the induction of tonic immobility is an aversive event. The experience of this aversive event could have adversely affected

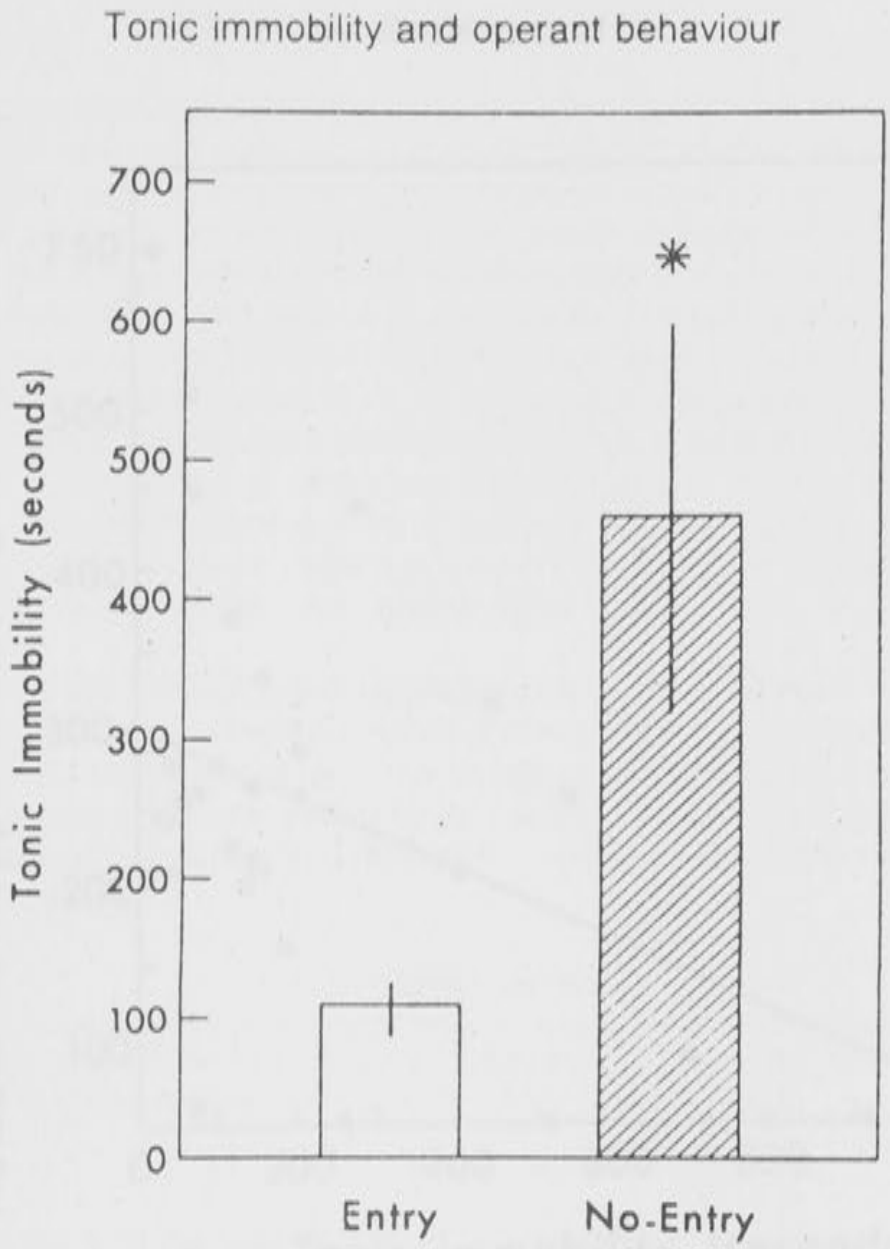

Figure 1. Mean tonic immobility (± standard error of the mean) for chickens that either entered (Entry) or did not enter (No-Entry) the food magazine on day 1 of operant training. Significantly different from Entry group, $P < 0.05$.

subsequent instrumental conditioning. Chickens were tested individually for tonic immobility on the floor of a BRS soundproof chamber with the door open for visual observation. The bird was manually restrained on its right side for 15 s. Tonic immobility was measured as the time in seconds from when the animal was released by the experimenter until it righted itself and rose to its feet. Additional induction attempts were given every 60 s if the previous one did not elicit tonic immobility. If the animal failed to elicit tonic immobility over five attempts it was given a score of zero seconds. The floor was cleaned and wiped with a weak vinegar solution between each test.

Results

Figure 1 shows the mean tonic immobility results of those birds that either entered the food magazine (broke photocell beam) at least once or did not (unbroken photocell beam) on day one of magazine training. The no-entry group

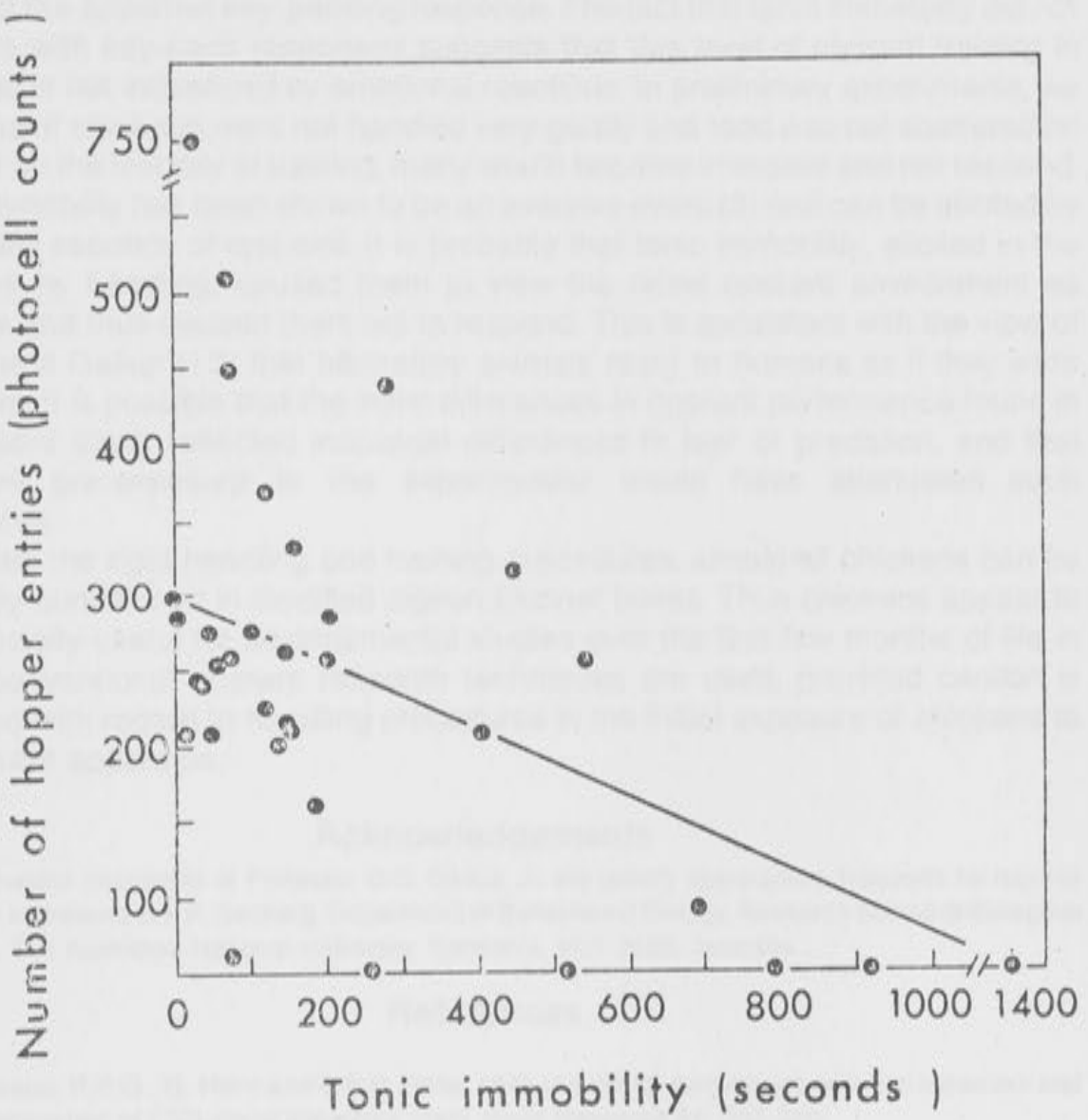

Figure 2. The relationship between number of hopper entries on day 3 of operant training and tonic immobility. The line of best fit is drawn ($\hat{Y}$ = -0.22 x +299.7).

showed significantly longer tonic immobility values than did the entry group (Mann-Whitney U = 57; n_1/n_2=19/11; $P < 0.05$). On day two of magazine training most birds were magazine trained. On day three (the first day of hopper training) there was a significantly inverse correlation between the mean number of magazine photocell counts and the duration of tonic immobility (Fig. 2: r = -0.44; df = 28; $P < 0.01$). On no subsequent days of training (autoshape or continuous reinforcement) were there significant relationships between key-pecking and tonic immobility.

Discussion

This study demonstrated that those chickens which responded less on the first days of magazine and hopper training also showed longer durations of tonic immobility. Tonic immobility, has been considered a good measure of fear in chickens (6). Therefore, fewer responses in the operant training probably reflected enhanced fear reactions or neophobia. In the present experiment, all animals

acquired the appettive key-pecking response. The fact that tonic immobility did not correlate with key-peck responses suggests that this level of operant training in chickens is not influenced by emotional reactions. In preliminary experiments, we found that if chickens were not handled *very* gently and food was not scattered on the floor on the first day of training, many would become immobile and not respond. Tonic immobility has been shown to be an aversive event (8) and can be elicited by only a few seconds of restraint. It is probable that tonic immobility, elicited in the chickens by handling, caused them to view the novel operant environment as aversive and thus caused them not to respond. This is consistent with the view of Suarez and Gallup (13) that laboratory animals react to humans as if they were predators. It is possible that the initial differences in operant performance found in the present study reflected individual differences in fear of predation, and that extensive pre-exposure to the experimenter would have attenuated such differences.

Given the right handling and training procedures, almost all chickens can be operantly conditioned in modified pigeon Skinner boxes. Thus chickens appear to be especially useful for developmental studies over the first few months of life in which conventional operant research techniques are used, provided caution is observed with regard to handling procedures in the initial exposure of chickens to the operant apparatus.

Acknowledgements

The helpful comments of Professor G.G. Gallup, Jr. are greatly appreciated. Requests for reprints should be addressed to P.R. Sanberg, Department of Behavioural Biology, Research School of Biological Sciences, The Australian National University, Canberra, ACT 2600, Australia.

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(Accepted: 24 February 1981)

Physiology & Behavior, Vol. 23, pp. 809–812. Pergamon Press and Brain Research Publ., 1979. Printed in the U.S.A.

Relationship of Some Open-Field Behaviors to Amygdaloid Kindled Convulsions in Wistar Rats[1]

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Received 16 May 1979

OSSENKOPP, K.-P. AND P. R. SANBERG. *Relationship of some open-field behaviors to amygdaloid kindled convulsions in Wistar rats.* PHYSIOL. BEHAV. 23(5)809–812, 1979.—Repeated low-intensity electrical stimulation (kindling) of the amygdala eventually produces convulsive behavior in animals. The present study examined the relationship between behaviors displayed in a novel open-field situation with behavioral characteristics of the kindled clonic convulsion (CC). Wistar rats were given two open-field tests and were subsequently kindled to clonic convulsions. A multiple regression analysis indicated that rats which urinated more often in the open-field tests tended to show longer latencies to CC onset. Thus, open-field urination was a significant predictor of latency to CC onset. It is suggested that an emotionality construct may be related to rate of kindling.

Kindling Amygdala Brain stimulation Convulsions Open-field test Epilepsy

THE PROCESS of repeatedly and intermittently applying a brief subconvulsive electrical stimulus to various limbic and cortical brain structures, called kindling, results in epileptiform activity and generalized convulsive seizure [4]. In the later stages of kindling the animal rears on his hind paws and bilateral clonic convulsions ensue.

Studies involving various strains of rats [13,20] and mice [8] have found that the rate at which animals kindle can vary greatly. A comparison of Tryon maze-bright and maze-dull rats showed that the bright strain required a greater number of amygdaloid stimulations with accompanying afterdischarges than the dull strain to manifest convulsion [20]. Zaide [20] suggested that greater behavioral inhibition in the bright strain could account for the observed differences. However, these two strains of rats also differ in terms of behaviors displayed in an open-field test [12,17], suggesting that these behaviors might correlate with rate of kindling as well. A number of studies have also suggested that the amygdala plays a role in the manifestation of "emotional" types of behavior in rats, e.g., [7, 9, 14, 18], and some of the open-field measures have been viewed as indices of emotionality in rats, cf. [5].

The present study attempted to determine the usefulness of some of the open-field measures as predictors of kindling related behaviors in Wistar rats.

METHOD

Animals

Twenty-four male albino Wistar strain rats weighing 350–400 g were individually housed with food and water available ad lib. The colony room was kept on a 12 hr light-dark cycle at a temperature of 20°C. All experimental measures were taken during the light portion of the light-dark cycle.

Surgical and Histological Procedures

All animals were anesthetized with sodium pentobarbital (50 mg/kg) and a bipolar electrode (Nichrome wire with trimel coating, 0.127 mm in dia., dipped in Epoxylite) was chronically implanted into the amygdala; the coordinates (with the incisor bar set at zero) were 0.5 mm posterior to bregma, 4.5 mm lateral to the midline, and 8.5 mm below the

[1]This research was carried out at the Department of Psychology, York University, Downsview, Canada. We would like to thank Dr. J. Gaito for the use of supplies and facilities.

[2]This research was supported by a National Research Council of Canada Postgraduate Scholarship to the first author. Please address all reprint requests to the first author.

skull. The side of the brain implanted with the electrode was randomly chosen for each rat. The electrodes were secured to the skull with four stainless steel screws and a covering of dental acrylic. Six or more days after surgery, the kindling procedures were initiated. Following the experimental procedure all electrode placements were verified histologically. The histological examinations indicated that all electrodes were within the amygdala.

Open-Field Tests

The dimensions of the square open-field apparatus were 76 cm on a side and 25 cm high. The inside of the open-field was covered with a dark green plastic sheet and the floor was marked off in 25 equal area squares by thin red lines.

Seven days prior to the implantation of the electrodes all rats received a free exploration trial in the open-field apparatus (Open-field Test 1). Each rat was placed in the center square along one side of the open-field and observed for 2 min. The number of squares entered by the four paws of the rat (ambulation), number of fecal boluses deposited in the arena (defecation), and the number of urination responses, were recorded during the 2 min test period. After removing the animal from the arena the floor was sponged over with tap water to remove residual odors. A second open-field test (Open-field Test 2) employing the same procedure, was given to all animals two days prior to the initiation of the kindling procedure (i.e., several days after the surgical procedure).

Behavioral Convulsion Development

The animals were stimulated three times daily (with an intertrial interval of at least one hour) by a 60 Hz sine wave of 100 μA intensity peak to peak, for 30 sec from a Lafayette sine wave stimulator. The rats' behavior was observed and a response was scored as a clonic convulsion (CC) only if the convulsion continued after the termination of the current; behavioral manifestations that terminated with current offset did not constitute a CC. If a CC response occurred, the latency to convulsion onset (time in sec between stimulus onset and initiation of the response) and the duration of the CC response (time in sec between response onset and termination), were recorded for each animal. If the animal aborted the CC at current offset no CC was scored. The duration or latency of the aborted CC response was excluded from data analysis. The experimental trials continued until each rat had displayed a CC response on six trials.

Data Analysis

Six open-field measures were used in the analyses: ambulation, defecation, and urination responses in Open-field Tests 1 and 2, respectively. Seven measures of the kindled convulsive responses were also obtained: (1) number of stimulations to first CC (trials to CC), (2) mean latency to CC onset for CC responses 2–6 (mean latency), (3) latency to CC onset for the first CC response (1st latency), (4) latency to CC onset for the sixth CC response (6th latency, (5) mean duration of CC responses 2–6 (mean duration), (6) duration of the first CC response (1st duration), and (7) duration of the sixth CC response (6th duration). These 13 variables (6 open-field and 7 kindling measures) were used to obtain a product-moment correlation matrix. Multiple regression analyses with forward stepwise inclusion of the open-field predictor variables using the Statistical Package for the Social Sciences computer program [11] were done on each of the kindling variables. Predictor variables were chosen for entry into the model on the basis of which variable had the largest squared partial correlation with the dependent (kindling) variable in each case.

RESULTS

Univariate Analyses

Means and standard deviations (SD) for the six open-field and the seven kindling related variables are given in Table 1. The correlation matrix for these 13 variables is presented in Table 2.

TABLE 1

MEANS AND STANDARD DEVIATIONS (S.D.) OF THE OPEN-FIELD AND KINDLING RELATED VARIABLES (n=24)

Variable	Mean	S.D.
Open-field: Test 1.		
1. Ambulation-1	25.96	13.26
2. Defecation-1	2.25	2.29
3. Urination-1	0.46	0.72
Open-field: Test 2.		
4. Ambulation-2	4.96	5.62
5. Defecation-2	1.79	1.41
6. Urination-2	0.38	0.49
Kindling related variables		
7. Trials to CC	10.58	4.73
8. Mean latency (sec)	8.58	5.30
9. 1st latency (sec)	11.63	6.61
10. 6th latency (sec)	8.25	5.15
11. Mean duration (sec)	28.63	6.06
12. 1st duration (sec)	22.79	9.55
13. 6th duration (sec)	28.54	6.87

The mean ambulation, defecation, and urination scores for Open-field Tests 1 and 2 (see Table 1) were statistically compared with correlated t-tests. Neither the defecation score means nor the urination score means differed significantly between the two tests, $t(23)=1.12$, $t(23)=0.47$, respectively. However, Test 1 ambulation scores were significantly larger than Test 2 scores, $t(23)=7.32$, $p<0.001$.

For the first open-field test the only significant intercorrelation for the three measures (Table 2) was a positive one between defecation and urination scores ($p<0.05$). None of the intercorrelations for the Open-field Test 2 measures were significant. Correlations of the measures from Open-field Test 1 with Test 2 resulted in a significant positive correlation between Defecation-1 and Defecation-2 ($p<0.01$). None of the other correlations were significant.

Intercorrelation of the kindling variables (Table 2) resulted in a number of significant correlations. These data have been discussed in a previous paper [16] and will not be dealt with further.

The correlations between the open-field variables and the kindling variables are shown in the bottom two rectangles of Table 2. Significant positive correlations were found between Urination-1 and mean latency ($p<0.05$) as well as 6th

TABLE 2

MATRIX OF CORRELATION COEFFICIENTS FOR THE OPEN-FIELD AND KINDLING RELATED VARIABLES (n=24)[1]

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13
Open-field: Test 1													
1. Ambulation-1	100												
2. Defecation-1	-01	100											
3. Urination-1	-25	51*	100										
Open-field: Test 2													
4. Ambulation-2	12	-01	-16	100									
5. Defecation-2	19	54†	-07	02	100								
6. Urination-2	-07	34	11	-18	12	100							
Kindling Variables													
7. Trials to CC	-04	28	30	11	-19	-21	100						
8. Mean latency	03	29	48*	-16	-07	44*	49*	100					
9. 1st latency	11	38	28	-19	09	51*	17	69†	100				
10. 6th latency	06	32	61†	-16	-03	22	50*	89†	54†	100			
11. Mean duration	04	03	03	-32	18	09	-56†	-05	11	03	100		
12. 1st Duration	-05	-09	05	-46*	04	28	-42*	21	07	30	69†	100	
13. 6th Duration	05	-30	-39	-41*	25	-08	-60†	-44*	-07	-50*	49†	14	100

[1]Decimals ommited. See text for a description of the variables.
*$p<0.05$
†$p<0.01$

latency ($p<0.01$). Similarly, significant positive correlations were obtained between Urination-2 and mean latency ($p<0.05$) and 1st latency ($p<0.05$). Significant negative correlations were found between Ambulation-2 and 1st duration ($p<0.05$) as well as 6th duration ($p<0.05$). None of the other correlations reached significance.

Multivariate Analyses

Only the latency and duration related variables showed significant multiple correlations with the predictor variables. For the latency related variables (see Table 3) all three measures of latency exhibited significant multiple correla-

TABLE 3

MULTIPLE REGRESSION COEFFICIENTS FOR THE LATENCY TO CC VARIABLES

Latency Variables	Open-Field Variables	Multiple R	Significance Level	Change in R^2	Significance Level
Mean latency					
	Urination-1	.479	.018	.230	.018
	Urination-2	.618	.006	.152	.034
	Ambulation-1	.643	.012	.032	.311
	Defecation-2	.654	.025	.015	.494
	Defecation-1	.655	.054	.002	.822
	Ambulation-2	.656	.101	.001	.864
1st latency:					
	Urination-2	.511	.011	.261	.011
	Urination-1	.561	.019	.053	.215
	Ambulation-1	.597	.029	.043	.262
	Ambulation-2	.604	.060	.008	.640
	Defecation-1	.611	.106	.009	.612
	Defecation-2	.615	.175	.005	.716
6th latency:					
	Urination-1	.612	.001	.374	.001
	Ambulation-1	.651	.003	.049	.194
	Urination-2	.671	.007	.027	.336
	Defecation-1	.676	.016	.007	.628
	Ambulation-2	.678	.036	.003	.767

tions with the open-field measures. Examination of which predictor variable produced the largest change in R^2 when added to the model, revealed that in all three cases one of the urination measures was involved (Table 3). For mean latency both Urination-1 and Urination-2 produced significant increases in R^2 and these two variables accounted for 38% of the variance in the mean latency. None of the other open-field variables produced significant increases in R^2 when added to the model.

For the duration related variables only the 1st and 6th durations showed significant multiple correlations with the open-field measures. Ambulation-2 plus Urination-2 correlated significantly with 1st duration ($p = 0.05$), and the linear combination of all open-field variables correlated with 6th duration ($p = 0.029$). Ambulation-2 contributed a significant increase in R^2 for both 1st and 6th duration ($p = 0.025$ and $p = 0.045$, respectively), and Urination-1 contributed a significant increase in R^2 for 6th duration ($p = 0.015$). Together, Ambulation-2 and Urination-1 accounted for 38% of the variance in 6th duration.

DISCUSSION

The major finding of this study was a positive association between open-field urination and latency to convulsion onset during the kindling procedure. That is, rats which urinated more often in a novel open-field situation also tended to have longer latencies to convulsion onset when kindled. This result is consistent with the previous finding that, compared to maze-dull rats, maze-bright rats take longer to manifest convulsions with amygdala stimulations accompanied by afterdischarges [20] in light of the demonstrations [12,17] that the maze-bright rats also defecate and urinate more often in a novel open-field. Although these earlier studies involved comparisons across strains, whereas the present results were obtained within one strain of rat, the direction of the relationship is the same in both cases. The present finding is also in agreement with a study by Martin and Hall [10]. These authors exposed rats bred for high levels of open-field defecation and urination (emotional) or low levels of defecation and urination (non-emotional) to an air blast and observed subsequent appearances of behavioral convulsions. The non-emotional rats had significantly more tonic-clonic convulsive seizures than the emotional rats [10]. Thus, low levels of defecation and urination in an open-field situation seemed to be characteristic of seizure prone rats. A final piece of evidence indicating that urination responses to novel or stressful situations may be related to seizure development in the rat comes from a study by Arnold, Racine and Wise [1]. In this study it was found that rats handled prior to the start of the kindling procedures required fewer afterdischarges for development of motor seizures compared to nonhandled rats. The nonhandled rats showed more defecation, urination, and vocalizations during the kindling procedures.

The findings of the present study suggest that a territorial marking emotionality construct is related to rate of kindling. Although the validity of open-field urination as an index of emotionality has been questioned [6,19], several studies with mice have shown that urination is an index of territorial marking [2,3]. Territorial marking has further been shown to be a first order manifestation of the higher order general factor of emotional stability, cf. [15]. The earlier demonstrations that the amygdala is involved in emotional types of behaviors in rats, e.g., [7, 9, 14, 18] together with the present results suggest that amygdala functions related to emotional behaviors may also be involved in the kindling process.

The importance of the present findings is the possibility of linking the mechanism(s) underlying kindling to some construct such as emotional stability, as well as predicting latency to CC onset on the basis of open-field urination levels. By correlating behaviors characterizing kindling to other behaviors such as those displayed in novel or stressful situations, we may be able to better speculate as to a possible underlying common mechanism.

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Brain Research, 200 (1980) 481–487

Spontaneously recurrent seizures after intracerebral injections of kainic acid in rat: a possible model of human temporal lobe epilepsy

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(Accepted July 3rd, 1980)

Key words: kainic acid — striatum — hippocampus — neurodegeneration — spontaneous seizures — metrazol-induced seizures — temporal lobe epilepsy

Intrastriatal injections of kainic acid in rats acutely induced repeated episodes of clonic convulsions. Spontaneously recurrent generalized seizures and a potentiation of the convulsant effects of pentylenetetrazol were then observed in most of the injected rats several weeks after surgery. In addition to marked loss of striatal neurons, limbic pathological alterations similar to those found in human temporal lobe epilepsy were observed in the brains of the kainic-acid treated rats. It is proposed that this preparation might serve as an animal model of human temporal lobe epilepsy.

Focal brain traumas, perinatal anoxia, or hyperthermia in early infancy can acutely induce serial convulsions, which are thought to be responsible for the later occurrence of spontaneously recurrent, partial or generalized seizures[3,17]. On histological examination, the brains of these patients frequently reveal a typical pattern of neuronal degeneration, neuronal loss, and astrocytic proliferation in the H1 and H3 fields of the hippocampus, with sparing of the H2 sector and the fascia dentata[2]. This hippocampal pathology is often associated with neuronal loss in the parahippocampal cortex, the amygdala, the deep layers of the neocortex, and the cerebellum[2]. The view[3,5,17,19,23] that metabolic alterations caused by the early episode of status epilepticus might be responsible for these pathologic changes is experimentally supported by the finding of a similar pattern of hippocampal damage after allylglycine-induced status epilepticus in subhuman primates[18]. Furthermore, clinicopathologic correlations appear to suggest that the altered activity of degenerating hippocampal neurons might play an important role in the pathogenesis of the spontaneously recurrent seizures[6,14,15]. An experimental evaluation of this hypothesis has, however, been hampered by the lack of an ontogenetically adequate model of medial temporal lobe epilepsy.

The data reported here indicate that intracerebral injections of kainic acid may provide such a model. Kainic acid (KA) has been intensively used recently on account

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of its potent neurotoxic properties[5,16,21]. Injections of KA into the rat striatum can cause neuronal destruction both locally and in extrastriatal areas, including hippocampus, cerebellum, pyriform cortex and amygdala[4,22,30]. The KA-induced pattern of hippocampal neurodegeneration is similar to that of human temporal lobe epilepsy, as it involves the CA1 and the CA3 fields, sparing both the CA2 field and the dentate gyrus. Here we report that KA striatal injections in rats acutely induce serial clonic convulsions, with later spontaneous recurrence of generalized seizures and potentiated convulsive response to pentylenetetrazol.

Ten male Wistar rats (Woodlyn Farms, Guelph, Ontario) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and 3 nmol of KA (Sigma Chemical Co.) dissolved in 0.5 μl of phosphate buffered isotonic saline, pH 7.4, were bilaterally injected into their dorsal striatum with a 34-gauge cannula over a period of 3 min, at the following coordinates: AP 8.4, ML $\pm$ 2.8, DV $+0.8$[12]. Ten control rats received bilateral intrastriatal injections of the vehicle solution only.

At no time after surgery did the control rats show seizures. In contrast, immediately after recovery from anesthesia all the KA treated rats showed repeated episodes of clonic jerking of the forepaws and bouts of body circling, which subsided after 4 – 5 h. Starting on day 35 after surgery, the rats were observed for 37 sessions of 10 min each, distributed over 42 days, with no more than one session per day. Locomotor and instrumental behaviors of the rats during these sessions have been reported elsewhere[25].

Generalized tonic–clonic seizures were observed in 7 KA treated rats between 35 and 77 days after surgery. Two KA rats manifested seizures once, another two twice, and the remaining rats in 3, 6 and 7 sessions respectively. No rat showed more than one seizure episode on any session. In addition to the tonic–clonic seizures 3 KA rats showed recurrent episodes of body circling and tail biting. These stereotyped behaviors were also exhibited by one KA rat that did not manifest tonic–clonic seizures. The seizures lasted 25–30 sec and their motor progression was similar to that of class 4–5 kindling induced seizures[26]. Typically, the rat stood in a semicrouched position with the head slightly flexed at the onset of the attack. Then it arched the trunk and stood up on its hindpaws, with the head extended. This tonic phase lasted 5–6 sec, and was followed by a series of rapid clonic jerks involving first one and then both forepaws. Although the rat usually maintained its balance during the attack, on occasions it fell on one side during either the tonic or the clonic phase. Postictally, the rat was hyperreactive, opposing attempts to handle it with either flight reactions or aggressive postures. With the possible exception of a chronically restricted food diet[25], no common precipitating condition could be identified, because the seizures occurred in a variety of environmental conditions, including the home cages, the maze and the waiting box used for the instrumental and the exploratory tasks, and the box with which the rats were carried from the colony room to the experimental room.

Light microscopy examination of frontal forebrain sections stained with either the Nissl or the Kluver–Barrera[11] method revealed bilateral loss of neurons and astrocytic proliferation in the core of both the dorsal and the ventral striatum, with a rim of apparently normal tissue remaining in the dorsomedial and the ventrolateral

TABLE I

Spontaneous seizures or stereotyped behaviors and limbic pathologic alterations in rats with striatal injections of kainic acid

Rat no.	*Episodes of seizures*	*Episodes of stereotyped behaviors*	*Hippocampal degeneration*	*Pyriform cortex degeneration*
1	—	—	CA1, CA3 bilaterally	None
2	6	—	CA1, CA3 bilaterally	None
3	7	—	None	Unilateral
4	3	5	CA1, CA3 bilaterally	None
5	—	9	CA1, unilaterally	Bilateral
6	2	—	CA1, bilaterally	Bilateral
7	2	4	CA1, bilaterally	Unilateral
8	1	4	CA1, bilaterally	None
9	1	—	CA1, unilaterally	None
10	—	—	None	None

periphery. The size of the striatal lesions was similar in all KA rats, extending 2.0–2.5 mm in the rostro-caudal axis, and including both the head and the body of the striatum, and the rostral pole of the globus pallidus. In addition to the striatal lesions, astrocytic proliferation and pycnosis or loss of neurons were detected in either the CA1 field, the CA3 field, or both, of the hippocampus of 8 KA rats, in the pyriform cortex of 4 KA rats, and in the juxtacallosal layers of the frontal cortex above the injection site in 3 KA rats. The morphology of these KA-induced lesions has been documented elsewhere[25]. All rats with spontaneous seizures or stereotypies, or both, showed damage to either the hippocampus or the pyriform cortex, or both (Table I). No such pathological alterations were found in rat 10, which showed no long-term epileptic phenomena. On the other hand, rat 1, which suffered neuronal loss in the hippocampus, showed no spontaneous seizures, suggesting that hippocampal neurodegeneration may not be a sufficient condition for at least the behavioral expression of chronic epilepsy. Alternatively, it is possible that this rat had seizures at times in which it was not observed.

The spontaneous seizures did not appear to depend on neocortical pathology, because such pathology was evident in only 3 chronically epileptic rats. On the other hand, a possible role of pyriform-cortex degeneration is suggested by the finding of spontaneous seizures in rat 3, which showed damage to the pyriform-cortex but not to the hippocampus. On the basis of the present data, a contribution of striatal pathology cannot be excluded: all KA rats, including those with spontaneous seizures, showed similarly severe striatal lesions, thus preventing any meaningful attempt to correlate striatal damage with epileptic behavior.

To examine further whether KA intracerebral injections chronically increase the effectiveness of seizure-precipitating agents, the convulsant response to pentylenetetrazol (Metrazol) was studied in another group of 16 rats, half of which were used as vehicle injected controls, and the other half treated with KA striatal injections similar to those performed in the first experiment. Forty-five days after surgery the rats recei-

TABLE II

Metrazol-induced convulsions in control and KA-neostriatal lesioned rats

Data represent means ± standard error of the means.

Item	*Controls*	*KA-lesioned*
Latency to first ictal response	621.6 ± 89.1	307.3 ± 49.9**
Latency to first generalized convulsion	763.3 ± 80.6	467.4 ± 77.8*
Duration of first generalized convulsion	73.6 ± 42.9	117.6 ± 31.0
Number of generalized convulsions	1.25 ± 0.18	4.63 ± 1.47*

* Significantly different from control group, $P < 0.05$ (two-tailed t-test).
** Significantly different from control group, $P < 0.01$ (two-tailed t-test).

ved Metrazol at a dose known to induce generalized convulsions in 97% of the rats (70 mg/kg, s.c.)[28] and then observed for 1 h in a test cage.

The progression of the seizures was similar to that of the spontaneous seizures observed in the first experiment, usually starting with intermittent myoclonic jerks of the body and merging into clonic or tonic–clonic convulsions of the forelimbs. Compared with the controls, the KA rats showed (Table II) significantly shorter latencies to the first ictal response (i.e. either a myoclonic jerk or, more rarely, a clonic convulsion), $t(14) = 3.3$, $P < 0.006$, significantly shorter latencies to the first generalized convulsion, $t(14) = 2.8$, $P < 0.02$, and significantly more episodes of generalized convulsions, $t(14) = 2.4$, $P < 0.05$. The incidence of the tonic component in the first convulsion was recorded, and did not appear to differ among groups: two controls and three KA rats showed such component. Three KA rats continued to show serial convulsions after the 1 h test period, with one of them dying of status epilepticus despite treatment with 10 mg/kg pentobarbital. Although the occurrence of spontaneously recurrent seizures was not systematically monitored in these rats, tonic–clonic seizures were witnessed in two KA rats about 2 h before Metrazol administration. In addition to extensive neuronal loss in the striatum, neuronal pycnosis and partial neuronal loss were found in the CA3–CA4 fields of the hippocampus of all KA rats, in the CA1 field of 4 KA rats (Fig. 1) either unilaterally or bilaterally, in the deep layers of the neocortex of 5 KA rats, bilaterally, and in the pyriform cortex of 3 KA rats unilaterally.

Recently, we have replicated the observation of spontaneous generalized seizures in several KA rats used for long-term behavioral studies, and have also observed clonic seizures in KA rats treated with doses of pilocarpine that are not convulsive for control rats[27].

Intracerebral injections of KA can thus be added to the short list of known experimental methods that produce both spontaneously recurrent generalized seizures and a chronic potentiation of the response to convulsant agents[13,21,29]. KA injections appear to have the advantage of simulating the proposed ontogenesis of human temporal lobe epilepsy[3,17], in which foci of limbic neurodegeneration caused by a prolonged episode of convulsive activity in early life can in turn play a role in the later recurrence of spontaneous seizures. With the present methods, however, the KA injec-

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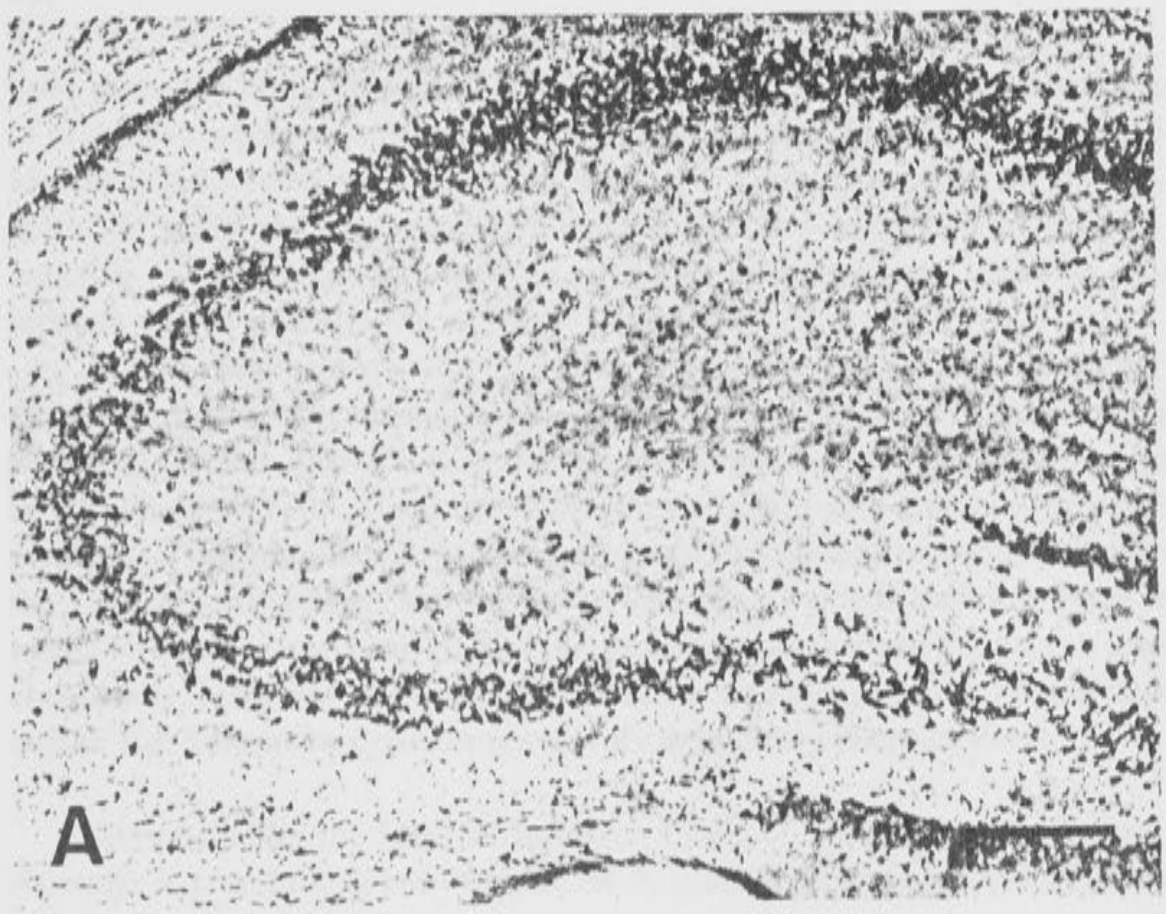

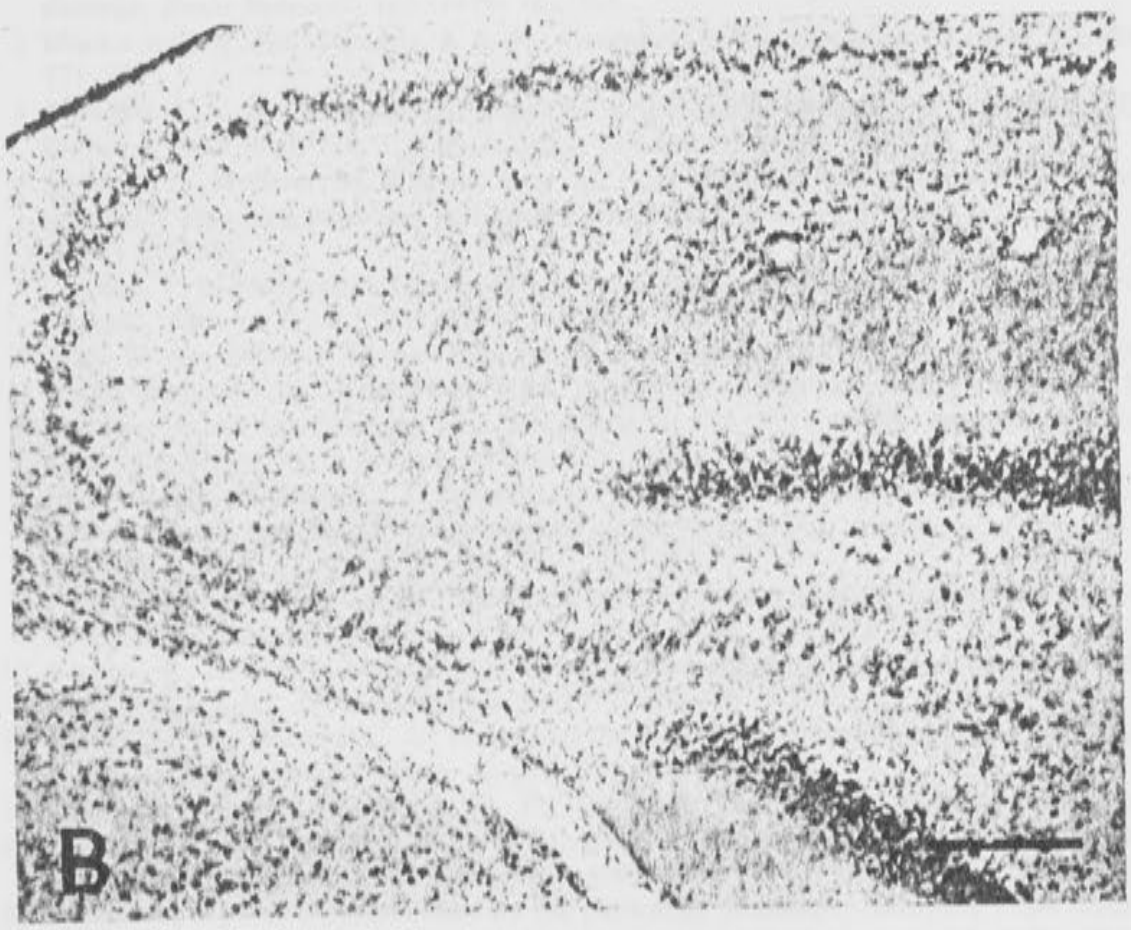

Fig. 1. A: control hippocampus. B: hippocampus of KA treated rat, showing loss of pyramidal neurons in fields CA1, CA3 and CA4. Nissl staining. Bars 200 μm.

tions damaged both limbic and extralimbic structures, especially the striatum. In light of previous studies, a contribution of striatal damage at least to the potentiation of the Metrazol-induced seizures might be expected[10]. Thus, to improve the specificity of the model of temporal lobe epilepsy further studies are warranted to examine whether selective, KA-induced degeneration of limbic structures can result in spontaneous seizures and in potentiation of the Metrazol-induced seizures.

It has been shown that systemic[22] or intraventricular[24] injections of KA, although apparently causing no damage to the striatum, acutely induce serial convulsions and result in a similar pattern of hippocampal and pyriform-cortex degeneration to that obtained with striatal injections. Pretreatment with antiepileptic drugs, such as diazepam[1,9] or homotaurine[8], has been shown to attenuate or completely prevent both the KA-induced seizures and the limbic damage. It would therefore be of interest to examine whether injections of KA through these non-striatal routes can also result in chronically recurrent seizures, and whether administration of antiepileptic drugs before KA administration can prevent its long-term epileptic effects.

Supported by the Medical Research Council. The excellent assistance of Stella Atmadja is gratefully acknowledged. M.P. is an M.R.C. postdoctoral fellow.

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Psychopharmacology 53, 207–209 (1977)

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Dose-Response Effects of Taurine on Some Open-Field Behaviors in the Rat

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Abstract. In two experiments albino rats were injected i.p. with various doses of taurine and their subsequent behavior in an open-field apparatus was observed. Increasing doses of taurine significantly decreased ambulation levels, increased latency scores, and increased thigmotaxis ('wall-hugging' behavior). In general, 50 mg/kg or more of taurine was required to produce significant changes in the dependent behavioral measures. The open-field behaviors of rearing and defecation were not significantly affected by the drug manipulation. The results of these experiments suggest that taurine may act as modulating or stabilizing agent in the central nervous-motor system rather than as a diffuse inhibitory agent.

Key words: Taurine – Open-field behaviors – Dose-response effects – Rats

The significance of taurine (2-aminoethanesulphonic acid) in nervous tissue is still not known with certainty. Behaviorally, i.p. injections of taurine (0.3–3.0 mg/kg) have produced a dose-dependent depression of habituated psychomotor activity in rats (Baskin et al., 1974). In mice, i.p. injections of large amounts (9–21.3 mmole/kg) of this amino acid resulted in decreased locomotor activity and decreased instrumental responding for food or water (Hruska et al., 1975). Intraventricular taurine administration also decreased locomotor activity and licking and grooming behavior in rats (Barbeau et al., 1975; Sgaragli and Pavan, 1972). Recently Persinger et al. (1976a) demonstrated that taurine (62.5–125 mg/kg) administered i.p. to postnatal-preweaning rats can have weak inhibitory behavioral effects on adult motoric behavior.

The present experiments investigated the effects of several dosage levels of taurine on a number of behaviors occuring in a novel open-field situation (for a review of the open-field test see Walsh and Cummins, 1976). The use of this multi-index open-field method allows one to construct a behavioral profile consequent to the drug manipulation (cf. Drew et al., 1972). Then, by comparing the effects of taurine upon the various behaviors measured, one could determine if this amino acid acts chiefly as a general inhibitory agent (by depressing all behaviors involving motor components and/or by increasing such autonomic responses as defecation), or as a more selective modulator or stabilizer in the CNS (by affecting only some of the behaviors).

MATERIALS AND METHODS

Thirty-five male albino Wistar rats (Woodlyn Laboratories) were used in Experiment 1 and 40 male rats in Experiment 2. Subjects weighed 450–500 g and 300–375 g, respectively, at the start of the experiments. Subjects in the first experiment had previously been used in a taste-aversion learning study involving an injection of LiCl. Subjects in Experiment 2 had been previously used in a learning study involving alleyway training to food reinforcement. Prior to the runway experiment, these animals were handled daily for 2–3 min over a 2-week period. More than 2 months elapsed between the taste-aversion study and Experiment 1, and 6 weeks between the alleyway study and Experiment 2. All subjects were individually housed, kept on a 12-h light-dark cycle (lights on 7.00 a.m. to 7.00 p.m.) and given ad lib. access to food and water during the experimental procedures described here.

Subjects in Experiment 1 were randomly assigned to 3 groups of 9 rats and one group of 8 rats. These groups were injected i.p. with saline, 3.0, 50.0, or 100.0 mg/kg of taurine. In the second experiment the rats were randomly assigned to 5 groups (8 rats/group) and injected i.p. with saline, 25.0, 50.0, 75.0, or 200.0 mg/kg of taurine. In both experiments the injection volume was 2 ml/kg.

After the injection each rat was coded and put back into its home cage for 1 h before being tested in the open-field apparatus.

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The open field was circular (85 cm diameter) and surrounded by a wall 30 cm high. The arena was flat black and the floor was marked off in 3 concentric circles and divided into 19 equal area segments. Five 100-W light bulbs 125 cm above the field provided illumination and the ambient noise level at field level was 55 ± 0.5 db. The open-field test was 3 min in duration and 4 behavioral measures were recorded: the latency to leave the initial square, the number of squares entered by the four paws of the rat, the number of rearing responses (lifting front paws off the floor and standing on hind legs), and the number of fecal boluses deposited in the arena during the test period. After removing the animal from the apparatus, the floor was sponged over with a weak vinegar solution to mask any residual odors. This procedure was repeated on 3 consecutive days. The subjects were run under a blind procedure so that the experimenter recording the open-field measures was unaware of the group designation of any given animal. Before data analyses were done, the latency measure was converted to a latency score by taking the reciprocal of the latency time. In addition, a thigmotaxis ratio (a measure of the 'wall hugging' behavior) was calculated by dividing the number of times a square in the inner two circles of the arena was entered by the total number of squares entered.

RESULTS

Each dependent variable was analyzed with a repeated measures design analysis of variance with the significance levels set at the 0.05 level of confidence. Post-hoc multiple comparisons were made with the Duncan multiple range test.

Figure 1 shows that increasing doses of taurine produced a decrement in ambulation in both experiments. Statistically, there were significant group differences in both Experiments 1 and 2 ($F = 4.067$, $df = 3/31$, $P < 0.025$ and $F = 2.738$, $df = 4/35$, $P < 0.05$, respectively). Post-hoc comparisons indicated that the 50 mg/kg group was significantly less active than the saline or 3 mg/kg groups in the first experiment and that both the 50 and 200 mg/kg groups were less active than the saline or 25 mg/kg groups in Experiment 2.

Significant group differences in latency scores were also found in both experiments ($F = 5.196$, $df = 3/31$, $P < 0.01$ and $F = 7.137$, $df = 4/35$, $P < 0.001$, respectively). In Figure 1 it can be seen that a decrease in the speed of leaving the initial square was a function of increasing taurine dosage level. Post-hoc tests showed that the 50 and 100 mg/kg groups were significantly slower than the saline or 3 mg/kg groups in Experiment 1, and the 50, 75, and 200 mg/kg groups were slower than the saline and 25 mg/kg groups in Experiment 2.

There were no significant group differences for the thigmotaxis ratio in Experiment 1 (possibly due to a large number of zero scores in the data), but in Experiment 2 the groups main effect was significant ($F = 2.931$, $df = 4/35$, $P < 0.05$). The multiple range test showed significantly less thigmotaxis for the saline

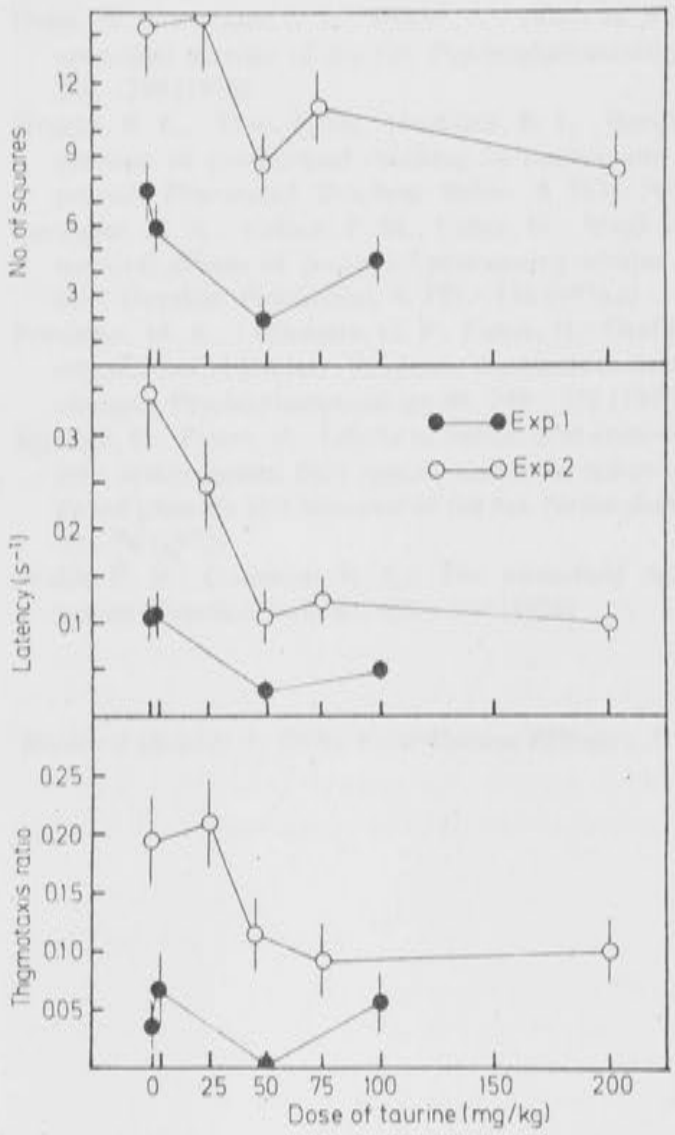

Fig. 1. Mean ambulation scores (*top panel*), latency scores (*middle panel*), and thigmotaxis ratios (*bottom panel*) as a function of taurine dose levels injected. Values for the first experiment are represented by the closed circles; for the second experiment by open circles. Error bars represent standard errors

and 25 mg/kg groups relative to the 75 and 200 mg/kg groups (see Fig. 1). In neither experiments were there any significant main effects or interactions for the rearing and defecation measures.

DISCUSSION

The present findings of decreases in ambulation resulting from increases in taurine injection levels is consistent with previous reports (Baskin et al., 1974; Barbeau et al., 1975; Hruska et al., 1975). Interestingly, no effects of taurine injections were found for the rearing measure. Ambulation and rearing responses are assumed to be two mutually exclusive motor responses (cf. Walsh and Cummins, 1976). The finding that taurine has an effect on only one of these motor behaviors seems to argue that taurine may not simply be a wide spectrum inhibitory agent, but rather may play a more selective role as a modulator or stabilizer in the CNS-motor systems. The failure to find significant effects of taurine on emotional behavior

(i.e., defecation) is also consistent with other studies (Persinger et al., 1976b). Our results suggest that a detailed study of the effects of taurine on the motor behaviors of rats in the open-field situation might provide some clues to the role of taurine in the CNS.

Acknowledgement. This research was supported by a National Research Council of Canada grant to Dr. N. I. Wiener and by a National Research Council of Canada Postgraduate Scholarship to the second author. We would like to thank D. Corey for technical assistance and Dr. M. A. Persinger for critical remarks about an earlier draft of this paper.

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Received October 5, 1976; Final Version February 3, 1977

Psychopharmacology 62, 97–99 (1979)

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Short Reports

Impaired Acquisition and Retention of a Passive Avoidance Response After Chronic Ingestion of Taurine

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Abstract. Oral administration of taurine (0.9%) in the drinking water resulted in impairment of acquisition and, to a lesser extent, retention of a step-down passive avoidance task in rats. No effect was found on spontaneous locomotor activity or habituation measured in photocell activity cages. There were also no differences observed in either the taurine-treated or control rats in their sensitivity to electric shock. These observations suggest that the administration of oral taurine may have adverse effects on inhibitory or memory functions.

Key words: Taurine – Learning and memory – Avoidance behavior – Locomotor activity – Rats

For many years taurine (2-aminoethanesulphonic acid) was considered to be a "non-essential" amino acid and was originally thought to be an end product of sulfur amino acid metabolism, which was readily excreted in the urine (Jacobsen and Smith, 1968). Recently, however, taurine deficiency has been implicated in many neuropathological conditions, such as epilepsy (Barbeau et al., 1975; Joseph and Emson, 1976; Konig et al., 1977), mental depression (Perry, 1976), and the alcohol withdrawal syndrome (Ikeda, 1977). As a treatment for taurine deficiency, oral taurine has been administered to patients with certain CNS disorders (Ikeda, 1977; Konig et al., 1977; Perry, 1976) because it has been demonstrated that taurine enters the CNS of neonatal and adult animals after such administration (Joseph and Emson, 1976; Persinger et al., 1976; Urquhart et al., 1974). Very little is known, though, about the side effects that oral taurine may have on normal behavior or in patients with CNS disorders. In a recent study, Persinger et al. (1976) found that taurine administered orally to adult rats resulted in significantly poorer acquisition of transient changes in a DRL (differential reinforcement of low rate of responding) operant paradigm. It has also been demonstrated that larger amounts of oral taurine increase fluid consumption in animals (Joseph and Emson, 1976). In the present study the effect of low concentrations of taurine (0.9%) on locomotor activity and passive avoidance learning and memory were studied to evaluate further the actions of taurine on normal behavior.

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Material and Methods

In experiment 1 twenty male Wistar strain rats (Woodlyn Farms, Guelph, Ontario) weighing about 200 g at the start of the study were housed in single stainless steel cages with free access to food (Purina rat chow which contains no taurine) and water (tap water). The rats were maintained in a 12-h light/dark cycle with temperatures between 21 – 25° C and humidity between 45 – 55%. Ten rats (experimentals) received chronically administered oral taurine (Fisher Scientific) by the addition of this amino acid (0.9% final concentration, 0.07 M) to the drinking water. Preliminary investigations, as well as reports from Persinger et al. (1976), indicated that this concentration did not significantly alter fluid consumption. Ten control rats received tap water only. Fluid intake was measured daily and water bottles were refilled at about the same time (10 a.m.) every 4 days. It has previously been shown (Joseph and Emson, 1976) that taurine is stable in tap water solution (97% remains after 4 days). Experimental rats were maintained on oral taurine before and during behavioral testing for a total of 29 days.

Twenty-two days following the start of the experiment, the locomotor activity of individual animals was assessed in photocell activity cages (BRS Foringer No. PAC-001) measuring 61 cm in diameter, with 43 cm high walls, an open top, painted black internally and being transected by six infrared photocell beams, interruption of which incremented electromechanical counters located approximately 9 m from the cages. Photocell beam interruptions were cumulated over 10-min periods and then recorded by an automatic printout counter (BRS Foringer No. POS 112) for 2 h. The environment contained white noise, temperatures ranging between 21 – 24° C, and was illuminated by four 100-watt bulbs located 3.6 m above the activity cages. The experiment was run for 4 h (beginning at 1 p.m.) on two consecutive days. Equal numbers of control and experimental rats were run during each 2-h testing session.

Two days following the locomotor activity measure. The rats were tested in a step-down apparatus consisting of a 27 × 27 × 30 cm high Plexiglas box with a 7.5 × 26.7 cm wooden platform shelf located to the side of the box and 9.4 cm above the grid floor. Each rat was given a small amount of electrode paste on its paws and then placed on the platform shelf. The latency of the initial step-down was recorded. Upon stepping off the platform and releasing a microswitch the rat received scrambled footshock (approximately 2 mA) until

returning to the platform. Footshock was supplied by a DC shock generator/scrambler (BRS Foringer No. SCS-003). Learning was measured in terms of the amount of time required for the rat to remain on the platform continuously for 3 min, and the number of descents the rat made until this criterion was reached. Twenty-four hours later, a retention test was conducted by placing the rat on the platform exactly as before and recording the latency to step-down measured to a maximum of 3 min.

Two days following the retention test, sensory detection thresholds to electric footshock were determined. The passive avoidance apparatus was modified by blocking off the platform shelf with a false wall. Before being placed on the grid floor, each rat received electrode paste to the paws. The animal was allowed to habituate to the apparatus for 3 min and then was presented with a series of inescapable footshocks (duration 0.5 s) of ascending intensities (0.25 – 3.0 mA). An intershock interval of 15 s was used. Shock was calibrated by using preset calibrations of certain intensities. The animal's response was rated as a flinch (any bodily movement), jump (hindpaws leave the grid) or vocalization. The threshold intensity was determined when the behavior occurred on three or more of the five presentations of that intensity. The passive avoidance and shock threshold experiments were run blind insofar as the experimenter (P.R.S.) did not know the identity of the animals. Another individual coded the animals prior to them being placed in the transport boxes, which held 5 rats each, and taken to the testing apparatus. The code was revealed at the end of the testing.

A subsequent replication study (experiment 2) using seven experimental ($\bar{x}$ wt = 522.6 ± 3.73 g) and seven control ($\bar{x}$ wt = 533.4 ± 3.09 g) rats is briefly reported, also. In this subsequent study oral taurine was given for 16 days prior to behavioral testing.

Statistical significance of the body weight, water intake, and locomotor data was analysed by a repeated measures two way analysis of variance (Winer, 1962). Student's *t*-test (Winer, 1962) and Mann-Whitney *U*-tests (Siegel, 1956) were used on the passive avoidance and shock threshold data.

Results

For experiment 1 there were no significant differences in the daily mean intake between tap water (39.2 ± 2.35 ml) and 0.9 % taurine solution (41.9 ± 2.35 ml). Experimental animals consumed a mean dose of 380 mg taurine/day or approximately 1.1 g taurine/kg/day. There were also no differences found in mean body weight of the groups over the course of the study. The mean weight at the start of the experiment for each group was 260.0 ± 8.43 g (controls) and 263.3 ± 3.68 g (experimentals); whereas the mean body weights were 378.4 ± 15.4 g (controls) and 376.7 ± 7.1 g (experimentals) as the start of the passive avoidance test.

No significant difference in spontaneous locomotor activity was found between control and experimental rats (group F ratio = 0.14, df = 1/18, $P > 0.10$) (Table 1). Habituation of the animals to the photocell cages over the test period also did not differ (interaction F ratio = 0.48, df = 11/198, $P > 0.10$).

Table 1 shows the results of the passive avoidance task. No significant differences were found on the initial step-down latencies recorded when the rats were first placed on the platform of the step-down apparatus. Experimental rats took significantly longer to reach the criterion of staying on the platform shelf for 3 min (t = 2.26, $P < 0.04$). However, there were no significant differences between groups on the number of step-downs to criterion. Thus, the experimental animals spent considerably more time on the platform between successive descents than did controls, however, they did not stay on long enough to reach criterion.

Table 1. The effect of oral taurine on passive avoidance acquisition and retention and sensitivity to electric shock. Data represent mean (± standard error of the mean) of ten animals in each of the control and taurine-treated groups

Item	Controls	Oral taurine
Spontaneous Locomotor Activity		
Photocell interruptions	1963.6 ± 277.4	2055.5 ± 162.4
Passive Avoidance Behavior		
Initial step-down latency (s)	7.69 ± 2.53	10.96 ± 3.68
No. of descents to criterion	3.40 ± 0.53	4.00 ± 0.63
Total time to criterion	251.4 ± 12.6	354.9 ± 46.7[a]
24-h Retest: Step-down latency (s)	136.2 ± 15.6	94.6 ± 24.9[b]
Shock Sensitivity Threshold		
Flinch (mA)	0.80 ± 0.09	0.75 ± 0.09
Jump (mA)	1.75 ± 0.15	1.55 ± 0.14
Vocalization (mA)	1.80 ± 0.18	1.65 ± 0.18

[a] $P < 0.04$ (two-tailed Student's t-test)
[b] $P < 0.07$ (two-tailed Mann-Whitney U)

The difference between the 24-h retention step-down latencies for control and experimental animals did not quite reach significance (U = 75, $P < 0.07$) in the present experiment. The control values were somewhat low compared to previous control results using this paradigm (see Sanberg et al., 1978); therefore an identical replication of this study was performed on a different set of animals. In this subsequent study (experiment 2) a significant retention deficit was found in oral taurine treated animals (controls = 147.6 ± 21.7 s, experimentals = 95.9 ± 24.3 s; U = 41, $P < 0.05$). Also, the total time to the acquisition criterion was significantly greater in oral taurine treated rats (controls = 242.4 ± 8.56 s, experimentals = 280.7 ± 16.78 s; t = 2.13, $P < 0.05$). No significant differences were found in initial step-down latency, number of step-downs to the acquisition criterion or locomotor

activity. There were no reliable differences in either experiment between the experimental and control animal's sensitivity to electric shock.

Discussion

The results demonstrate that the acquisition and, to a lesser extent, retention of a passive-avoidance task can be adversely influenced by the ingestion of 0.9% taurine solution. Previous investigations have shown that taurine administered by other routes produces a dose-related depression of various measures of locomotor activity (Barbeau et al., 1975; Sanberg and Ossenkopp, 1977). It is possible that motor deficits such as these may account for the passive avoidance impairments; however, in the present animals no significant differences were found between the oral taurine-treated and control groups on spontaneous locomotor activity as measured in photocell cages, or on the initial step-down latency recorded when the rat was first placed on the platform of the step-down apparatus. The discrepancy between our locomotor activity results and those of previous reports using other means of administration, may be due to the fact that oral administration causes no intraperitoneal irritation, and less stress and handling artifacts which can confound this measure. Adaptation of the animals in terms of gross motor activity over the relatively long period of taurine administration may have also occurred. In support of the present results, Ossenkopp (personal communication) recently demonstrated that 0.9% oral taurine does not alter open-field activity. It is also possible that a drug-induced reduction in sensitivity to footshock was responsible for the deficits observed in passive avoidance behavior. This appears unlikely, however, since no differences were found in either flinch-jump or vocalization shock thresholds.

Taurine has come under much interest recently as a potential treatment for various neurogenic conditions where a deficiency of taurine has been proposed (Barbeau et al., 1975; Ikeda, 1977; Joseph and Emson, 1976; Konig et al., 1977; Perry, 1976). Taurine appears to enter the brain when administered orally to neonatal and adult animals (Joseph and Emson, 1976; Persinger, 1976; Urquhart et al., 1974), and increases plasma levels when given to humans (Urquhart et al., 1974). It is not known at present what possible side-effects oral taurine may have when used as a therapeutic agent but recent work with animals, as well as the present results, indicate that taurine can exert some effects (possibly detrimental) on the behavior of the normal organism. The mechanisms for these behavioral effects are not known. Taurine may be having a peripheral effect since nearly 75% of taurine is located within muscles (Jacobsen and Smith, 1968); alternatively, if taurine is a neurotransmitter (Barbeau et al., 1975) it may be acting through specific "taurinergic" receptors. Taurine has also been implicated as a neuromodulator (Barbeau et al., 1975; Nakagawa et al., 1977) and has been shown to inhibit the release of acetylcholine and noradrenaline from various brain areas (Nakagawa et al., 1977). This is interesting in the context of the present experiment, since it is known that reduction of cholinergic activity can impair passive-avoidance learning and memory (Fernández Sanblàncat et al., 1977; Sanberg et al., 1978).

Acknowledgements. The authors appreciate the assistance of Dr. E. G. McGeer, W. Staines, K. E. Buxton, S. Atmadja, D. A. Sanberg, Dr. A. Benjamin, Dr. T. L. Perry and Dr. K.-P. Ossenkopp in the present study. This research was supported by the Medical Research Council of Canada.

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Received May 11, 1978, Final Version December 12, 1978

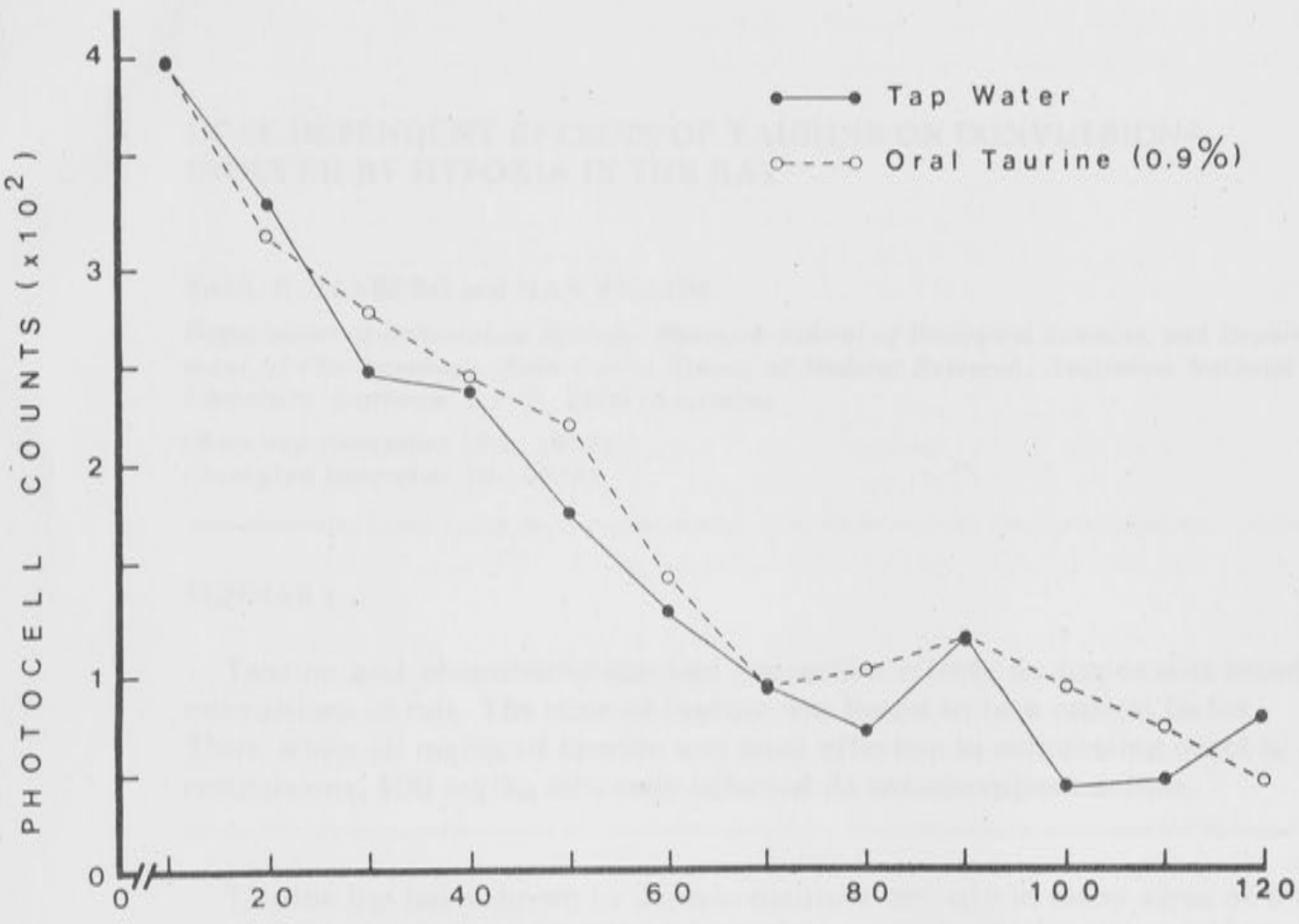

Figure 1. Locomotor activity in controls and rats treated with oral taurine. Values are mean photocell beam interruptions per 10 minutes for a 2 hour period.

Neuroscience Letters, 16 (1980) 297—300
Elsevier/North-Holland Scientific Publishers Ltd.

DOSE-DEPENDENT EFFECTS OF TAURINE ON CONVULSIONS INDUCED BY HYPOXIA IN THE RAT

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(Received November 29th, 1979)
(Accepted December 7th, 1979)

SUMMARY

Taurine and phenobarbitone had protective effects on hypoxia-induced convulsions in rats. The dose of taurine was found to be a critical factor. Thus, while 50 mg/kg of taurine was most effective in suppressing onset of convulsions, 100 mg/kg adversely affected its anticonvulsant action.

Taurine has been shown to depress neuronal activity in many areas of the central nervous system [2,10]. A reduction in levels of this amino acid in human and experimentally-induced epileptic foci [2,19,22], and the fact that taurine is an anticonvulsant in animals [1,2,9,17,22], has suggested a role for this amino acid in the pathophysiology of epilepsy [2,22]. Recent investigators, however, have reported conflicting evidence [4—6,13,20]. While many reports have demonstrated antagonism of seizure activity by taurine in a variety of experimental conditions [1,2,9,17,22], others have either failed to confirm these [6,14,18] or found a lack of effect on different convulsive disorders in animals [1,5—7]. We have compared the effects of various doses of taurine and phenobarbitone, a known anticonvulsant, on hypoxia-induced convulsions to evaluate further the role of taurine as an anticonvulsant.

Male Wistar hooded rats, from the Department of Psychology, A.N.U. animal colony, weighing between 250—350 g were used. The animals were housed in groups of five, kept in a 12 h light/dark cycle, and given ad lib. access to food and water. Separate groups of animals were given various doses of sodium phenobarbitone or taurine, dissolved in 0.9% NaCl solution, coded, and injected intraperitoneally in a volume of 1 ml/kg. After injection the rats were put back into their home cage for 1 h before being tested for hypoxia-induced convulsions. Rats were subjected to hypoxia as described elsewhere [12]. Briefly, they were placed individually into a 10-litre transparent plastic bag which had been freshly filled with 2% O_2 in N_2. The time

to the onset of clonic-tonic convulsions was recorded. The animals were removed after they began convulsing or if two minutes had elapsed. Results were analysed using probit analysis [11].

The critical response time (CRT) for the onset of clonic-tonic convulsions in a groups of rats treated with 0.9% NaCl solution alone was calculated as the mean response time plus two standard deviations ($\bar{X}$ = 65.4 sec, S.D. = 14.14 sec, CRT = 93.7 sec, n = 24). Animals in the taurine and phenobarbitone groups which failed to convulse within the CRT were designated as non-convulsant. The dose of taurine which protected 50% of animals from hypoxic convulsions (ED_{50}) was calculated using the groups treated with 6.25—50.0 mg/kg of taurine (Table I). The ED_{50} ± S.E.M. was 11.39 ± 1.72 mg/kg. Increasing the dose of taurine to 100 mg/kg afforded less protection against hypoxic convulsions, with only 40% of animals not responding within the CRT. Phenobarbitone, in doses of 25.0 and 50.0 mg kg protected 50% and 90% of the animals, respectively, against hypoxic convulsions (Table I).

The dose-dependent anticonvulsant effects of phenobarbitone on hypoxia-induced convulsions are consistent with previous findings [8]. Taurine also had a protective effect on hypoxic seizures, but dosage appeared to be a critical factor. Thus, 100 mg/kg taurine produced less effect than the lower doses used; with 50 mg/kg proving most effective. In fact, there was a tendency for those animals in the 100 mg/kg group that did convulse within the CRT to have shorter latencies to convulsion than controls. In support of this, Gaito [15] showed that although at both 100 mg/kg and 50 mg/kg doses, taurine produced a slight protective effect on the development of kindled convulsions, 'only with the latter dose was the difference statistically significant.' Furthermore, Carruthers-Jones and Van Gelder [9] recently showed an 'adverse effect of large doses of taurine' on the amino acid pattern of cobalt-induced epileptogenic foci. Therefore, it seems likely that differences

TABLE I

EFFECT OF PHENOBARBITONE AND TAURINE ON THE ONSET OF HYPOXIA-INDUCED CONVULSIONS

Dose (mg/kg)	N	% of animals not responding within CRT
Phenobarbitone		
25.0	10	50.0
50.0	10	90.0
Taurine		
6.25	11	45.4
12.5	15	55.3
25.0	15	66.7
50.0	15	73.3
100.0	15	40.0

in dosage are a major factor in the conflicting reports on the anticonvulsant action of taurine [1,2,5—7,9,14,17,18,22].

The mechanism of action of taurine in protecting against hypoxic convulsions cannot be determined from the present study. While taurine has been shown to protect against a variety of experimentally-induced seizures [1,2,9, 17,22], several non-specific mechanisms may contribute to its pharmacological action. These include depression of neuromuscular transmission [3,16] and a dose-dependent reduction in locomotor activity [21].

ACKNOWLEDGEMENTS

The authors are grateful to Professors R.F. Mark, G.A.R. Johnston, D.R. Curtis, Dr. W.P. Bellingham and Professor C. Allweis for helpful comments throughout the course of the study. The authors are both A.N.U. Research Scholars.

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Corticosteroids and Chorea

To the Editor.—In an article in the ARCHIVES (35:53-54, 1978), Green reported improvement after treatment with corticosteroids in the conditions of eight consecutive patients suffering from Sydenham's chorea (SC). Green suggested that the beneficial effect of corticosteroids on SC may involve alleviating "a mild inflammatory reaction of small vessels most likely involving the caudate-putamen complex." Another possibility worth consideration is that his results reflect suppression of an autoimmune mechanism. Recent work by Husby et al[1] suggests that, in SC, an "antineuronal" antibody is present that reacts with the neuronal cytoplasm of human caudate. It is possible that corticosteroids, by reducing production of such an antibody, might bring about a favorable therapeutic response. If this is true, a similar approach might be considered for the treatment of Huntington's disease (HD), inasmuch as there have also been reports of an autoimmune response in this disorder.

McMenemy in 1961[2] first reported the presence of a possible immunological reaction in HD. Husby et al[1] reported the presence of an anticaudate neuronal antibody and Barkley et al[4,5] showed that a cellular immune response of lymphocytes exists in the presence of brain tissue in patients with HD, but Williams et al[6] could not confirm the existence of a cell-mediated immune reactivity that is specific or unique for antigens in the brain tissue of patients with HD.

We have administered, to two women (aged 52 and 53 years) with advanced HD, prednisone in dosages comparable to those used by Green, ie, 30 to 45 mg/day. Neither patient showed any improvement in mood or in gross movement, although one showed a mild improvement in a simple hand dexterity test over a two-week period. The test was discontinued after two months in one patient and three months in the other to avoid complications. Although these results do not support the autoimmune theory for HD and the literature reviewed earlier is controversial, the possibility still deserves pursuit, possibly with less advanced cases of HD. If part of the chronic degenerative process is due to antibodies, corticosteroid treatment might inhibit progression of the disease, but it would be unlikely to induce any dramatic reversal of established symptomatology.

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